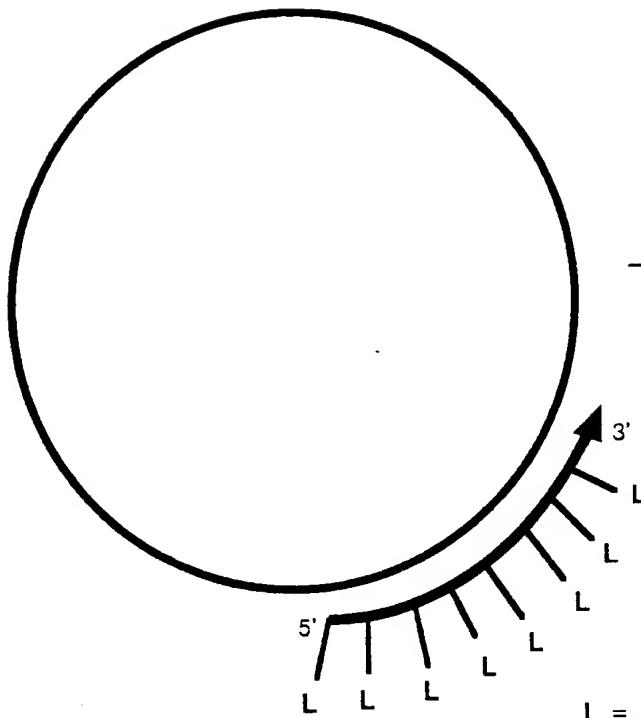


(a)

 $L = \text{Lactyl moiety}$

(b)

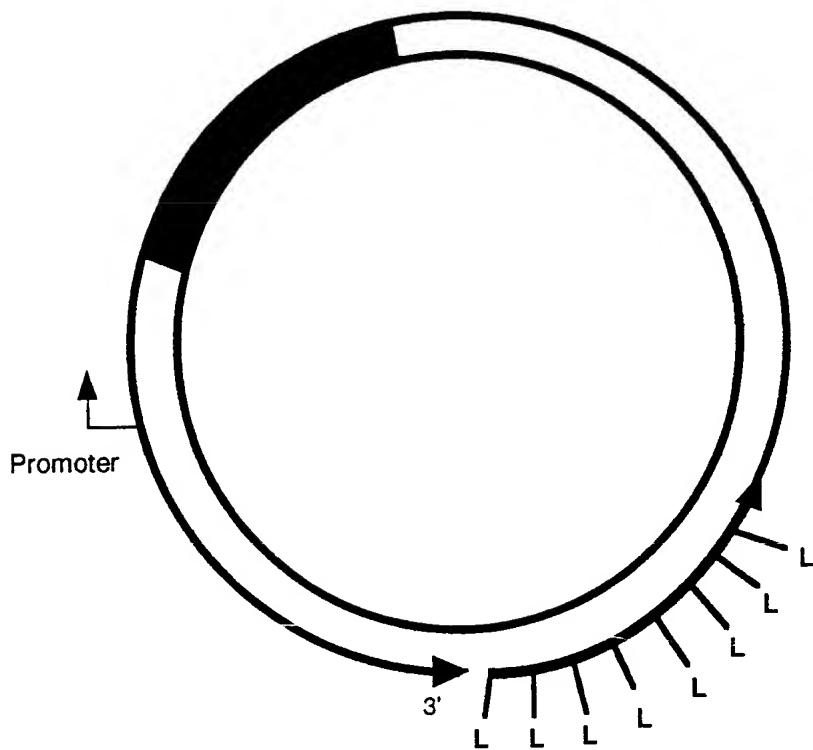


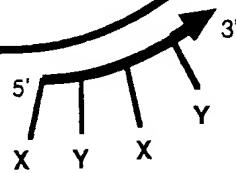
Figure I

Attachment of Ligands Through Primer Region

(a)

X = Nuclear Localisation Signal

Y = fusogenic peptide



(b)

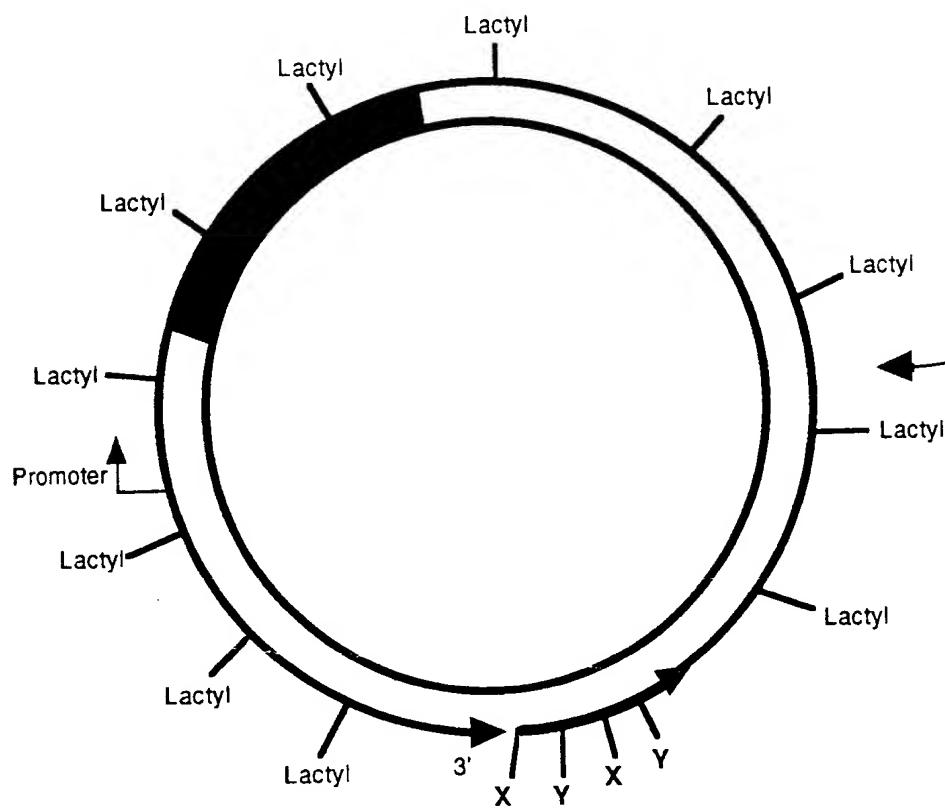
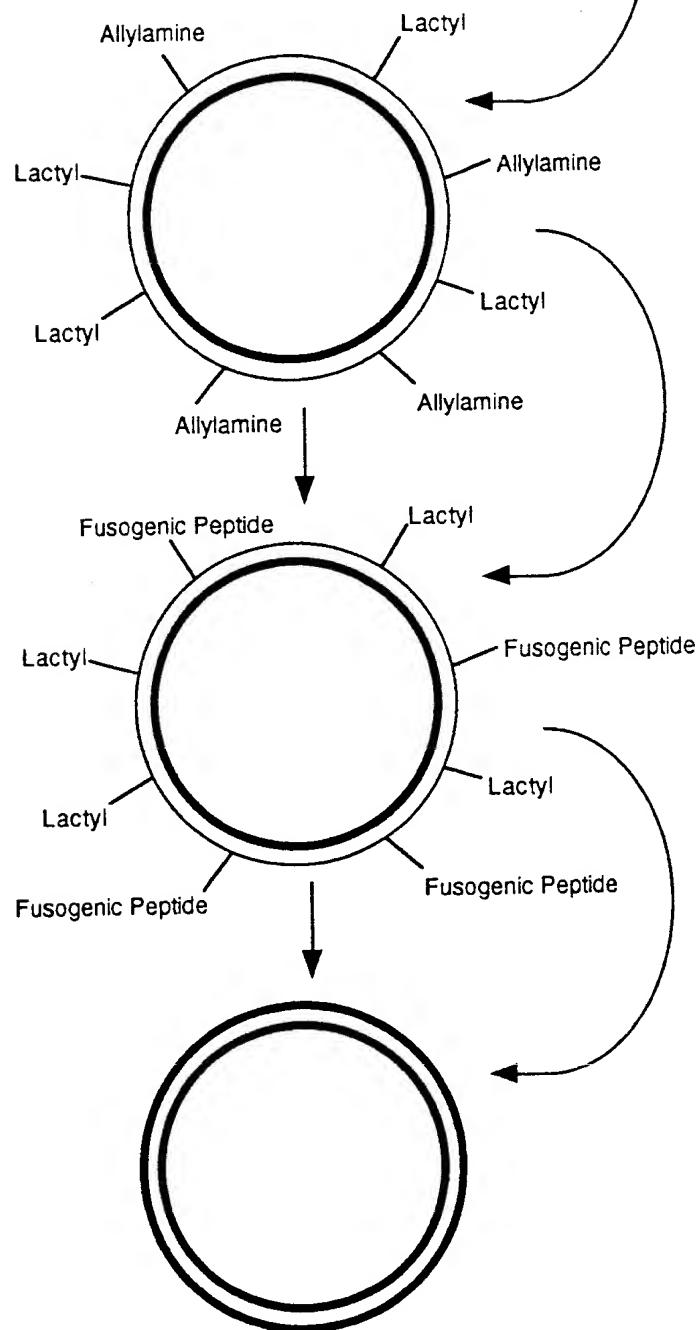


Figure 2
Attachment of Ligands by Incorporation of
Modified Nucleotide Precursors

— = DNA
— = RNA

Synthesis of RNA using lactyl-UTP and Allylamine-UTP precursors



Attachment of fusogenic peptide through allylamine linkage

- 1) attachment of construct to cell surface
- 2) endocytosis of construct
- 3) release of construct from endosome by means of fusogenic peptide
- 4) Elimination of RNA moieties by RNaseH
- 5) Synthesis of complimentary DNA strand

Figure 3
Incorporation of Ligands through Modified Ribonucleotides

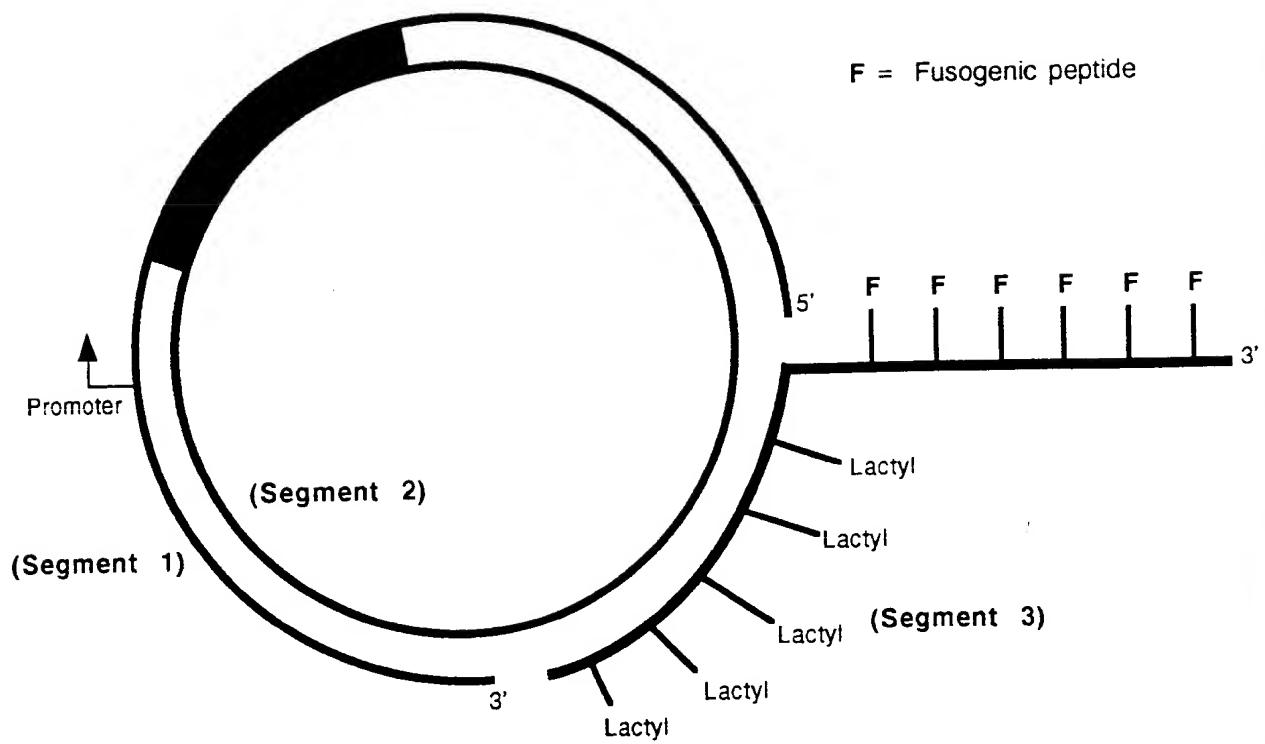


Figure 4
Attachment of Ligands through a 3' tail

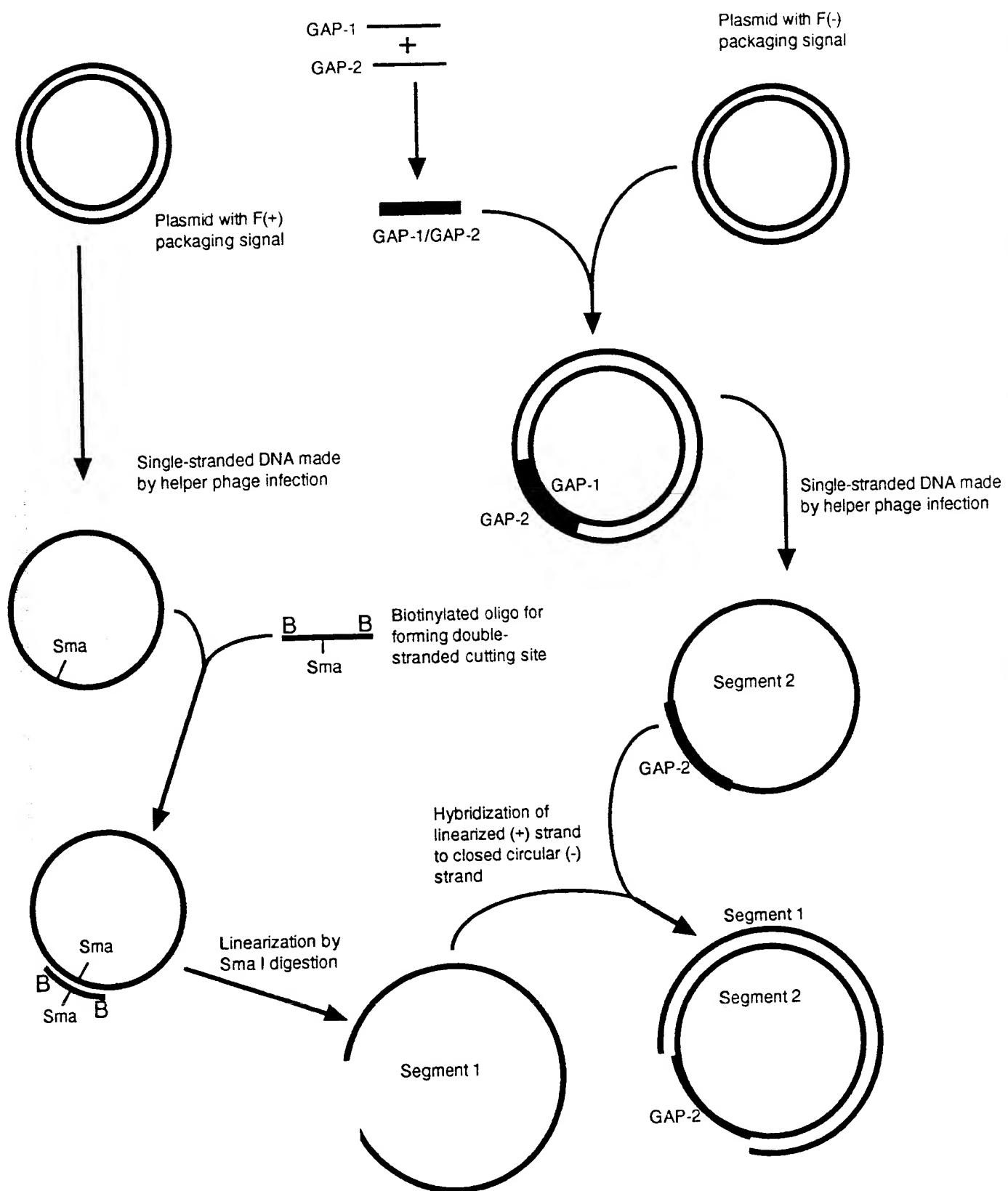
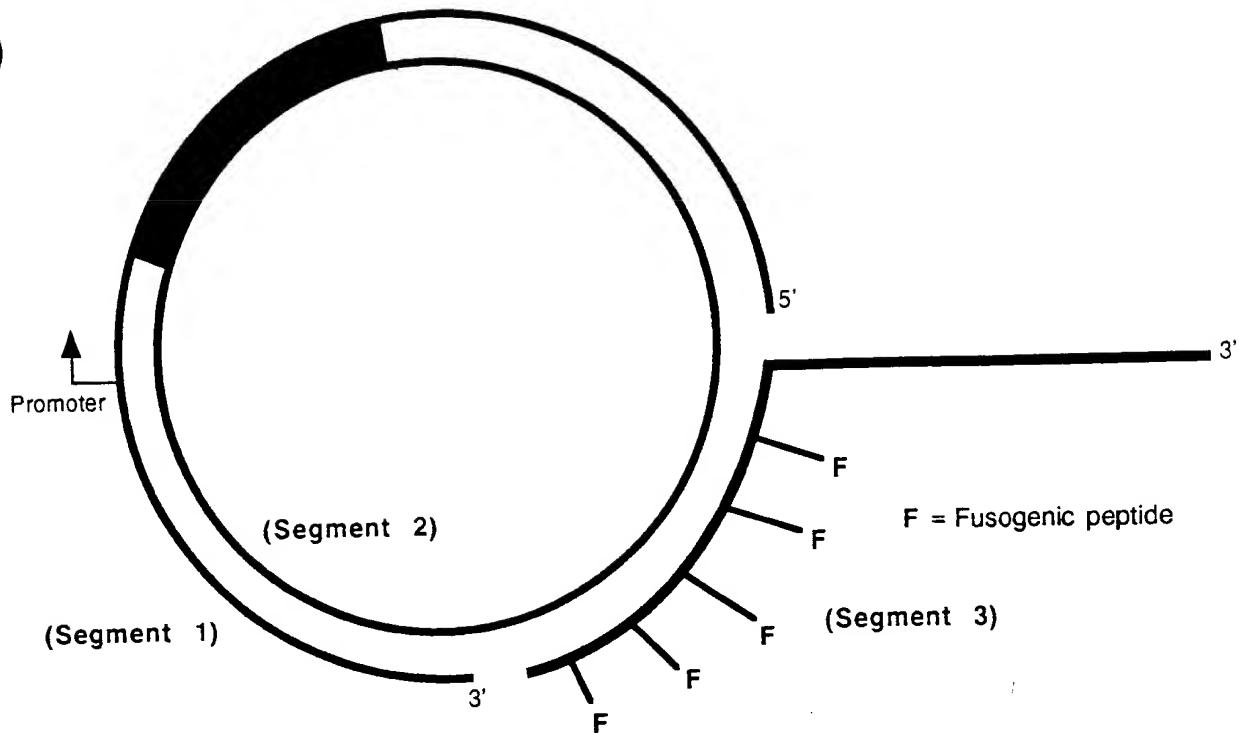


Figure 5
 Preparation of Gapped Circle

(a)



(b)

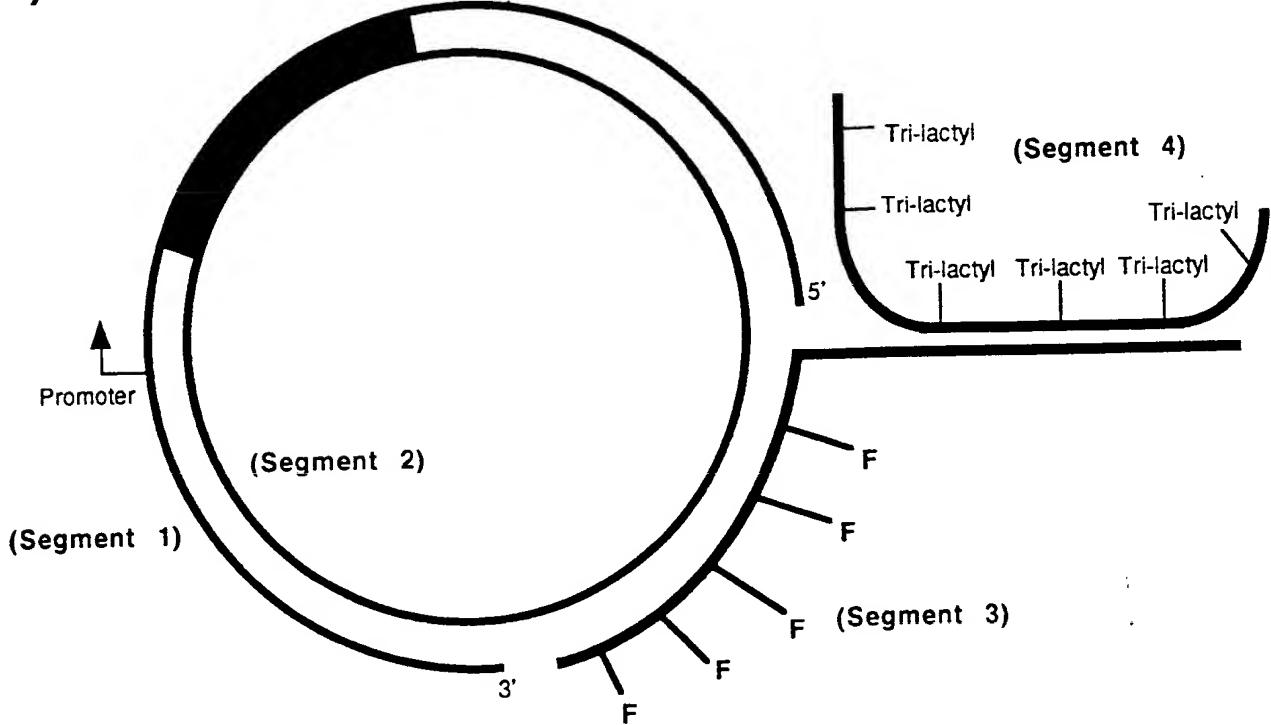


Figure 6
Attachment of Ligands through hybridization to a 3' tail

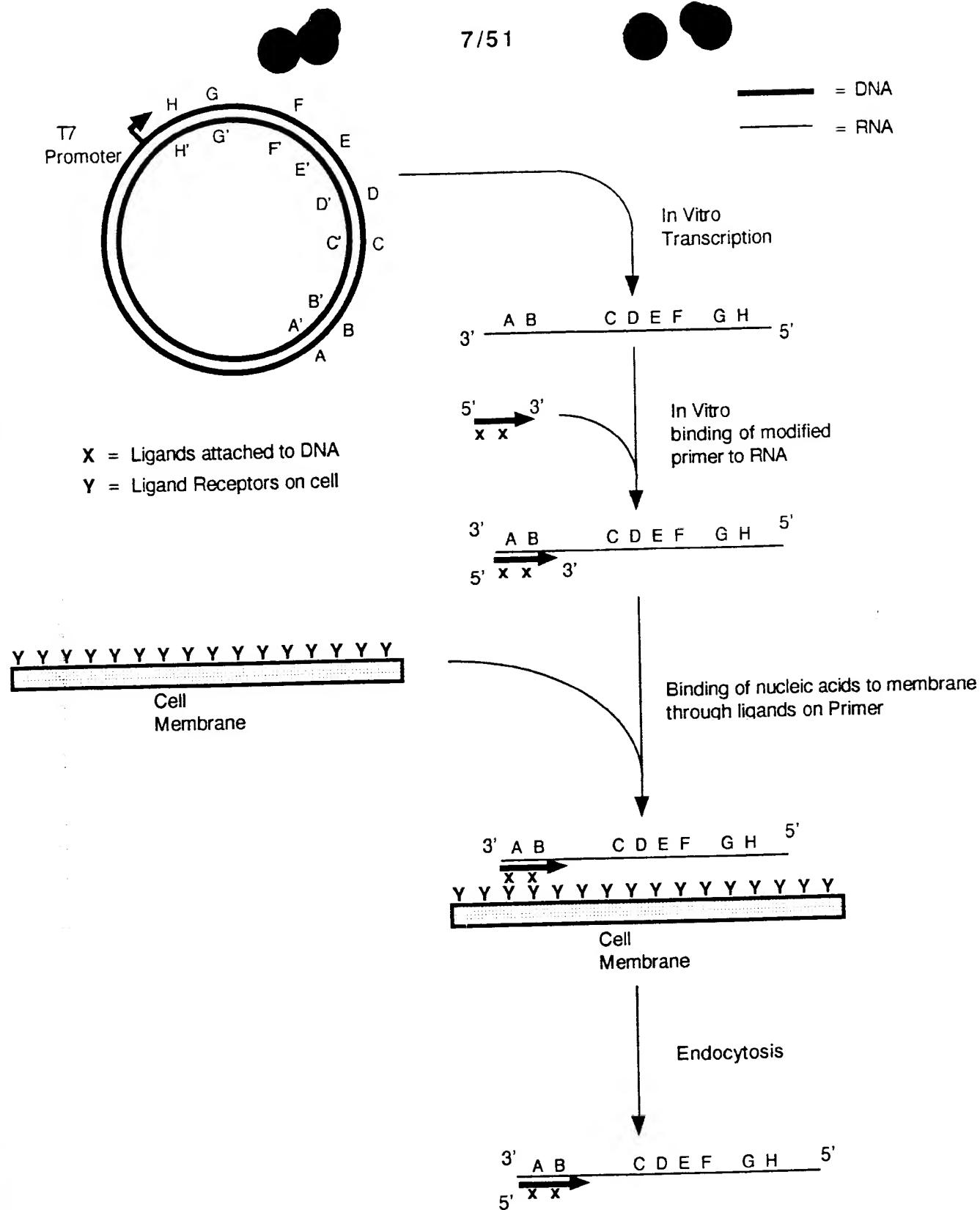


Figure 7

RNA with Ligands on Primer

(Continued in Figure 8)

Continued from Figure 7

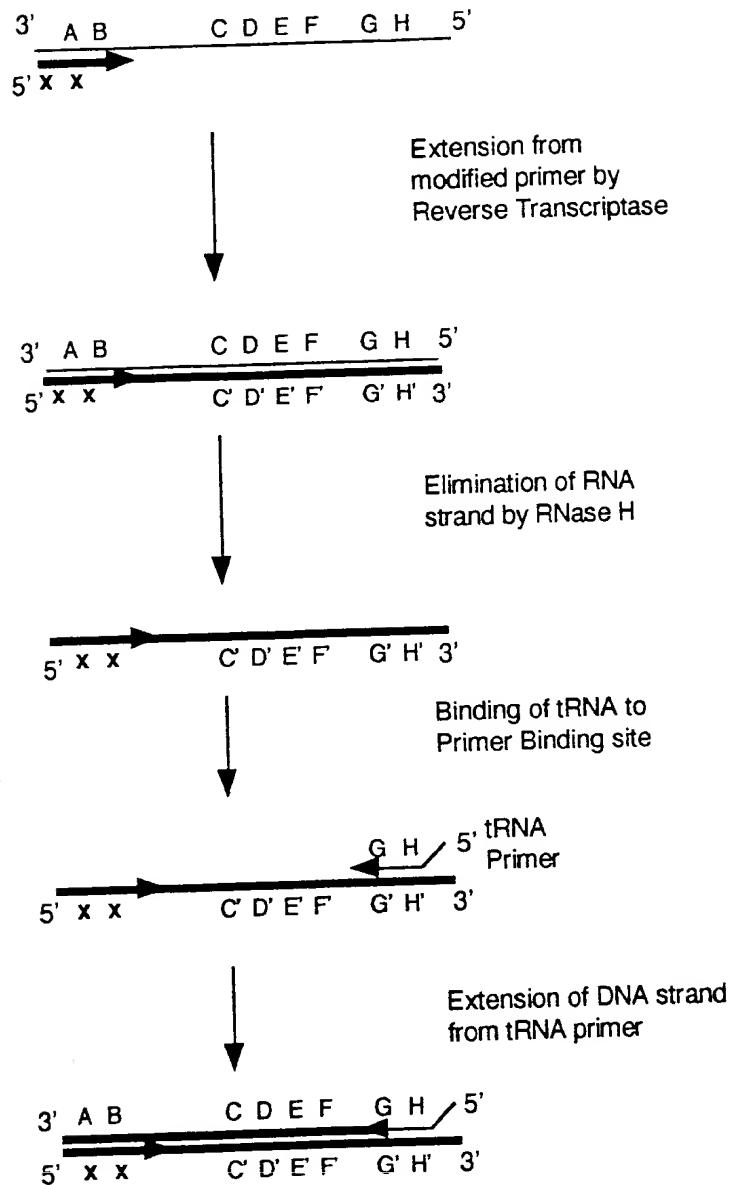
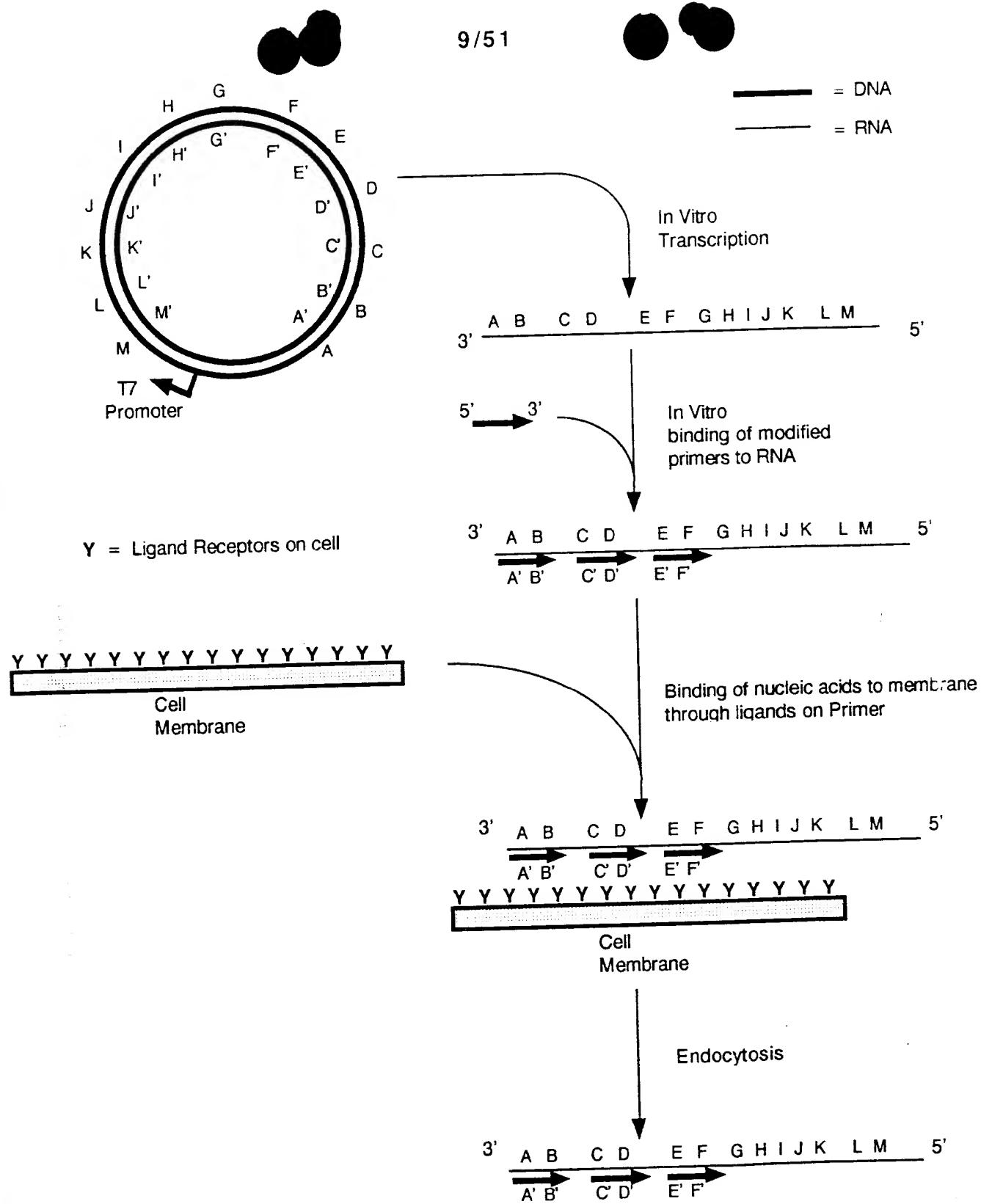


Figure 8
RNA with Ligands on Primer (Continued)



(Continued in Figure 10)

Figure 9
RNA with Ligands on Multiple Primers

Continued from Figure 9

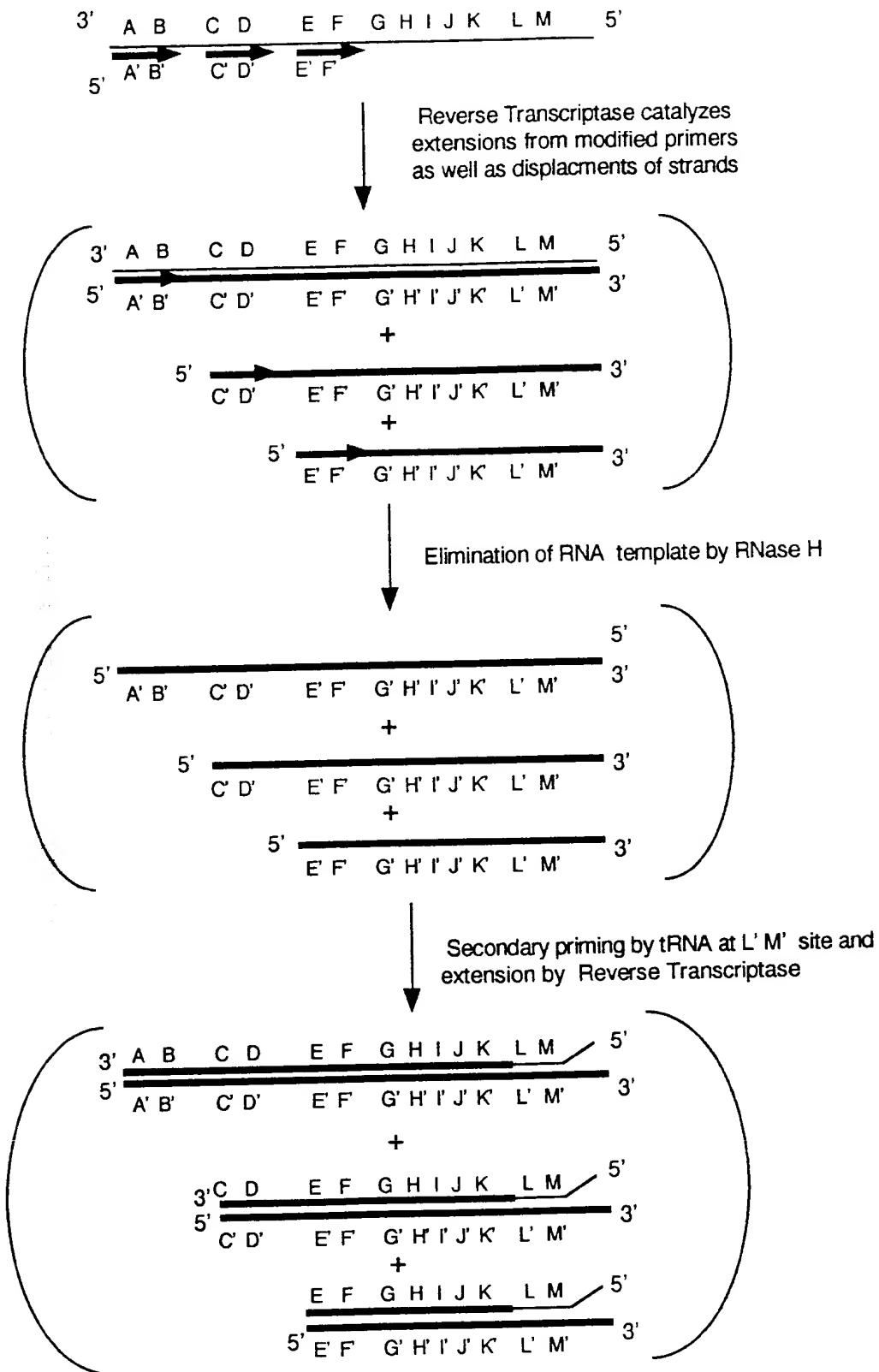


Figure 10
RNA with Ligands on Multiple Primers (Continued)

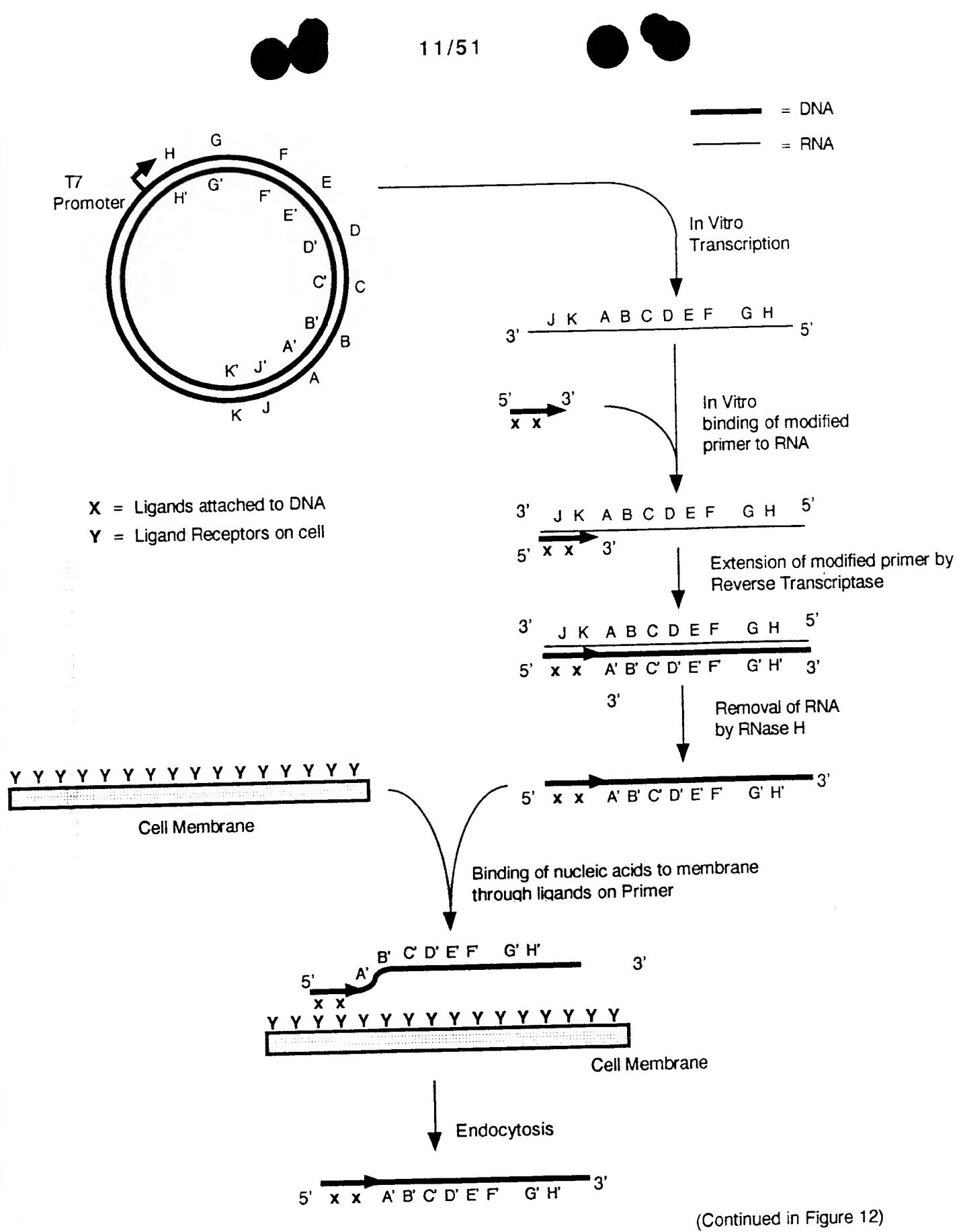


Figure 11
Single-stranded DNA with attached Ligands

Continued from Figure 11

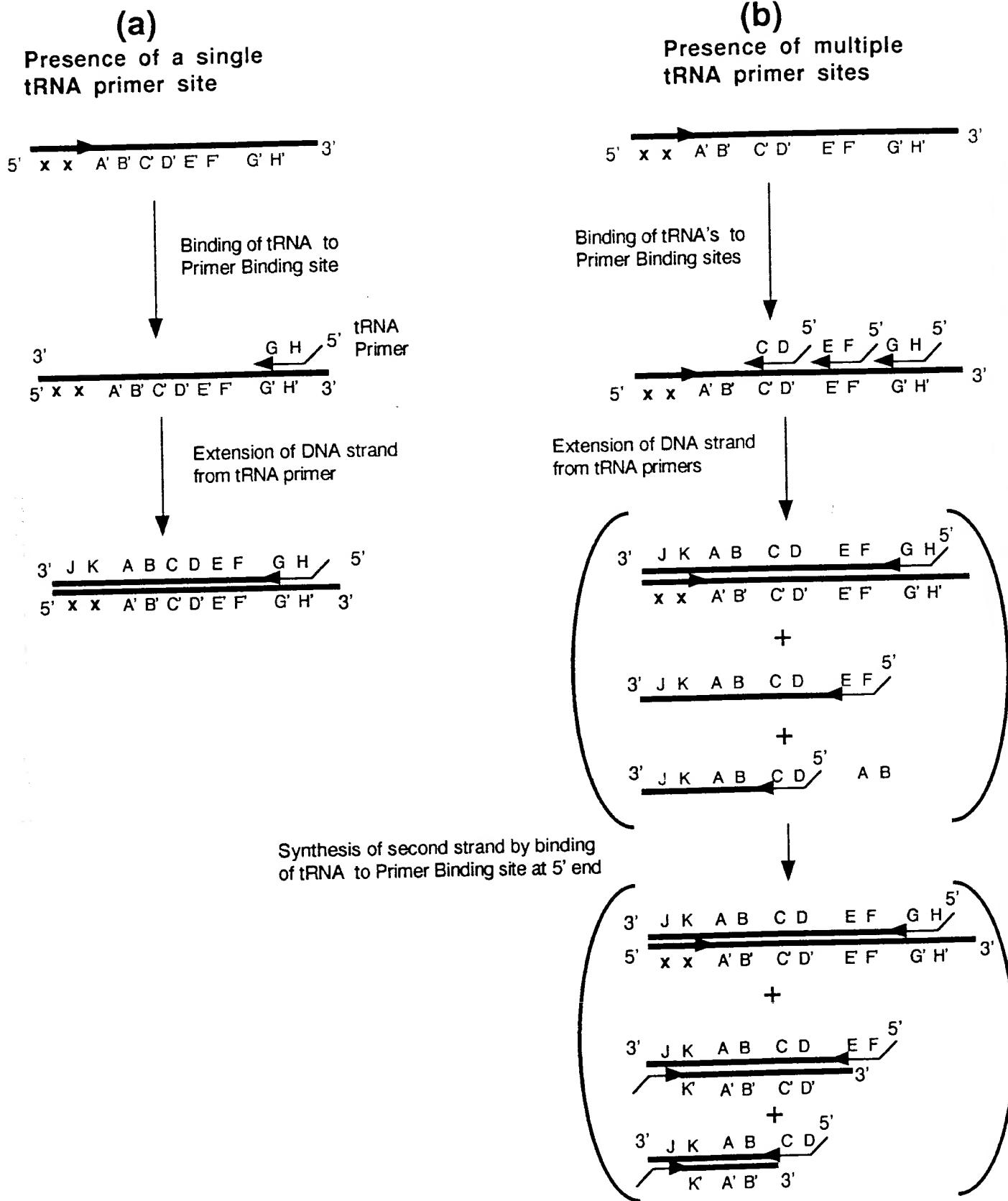


Figure 12
Single-stranded DNA with attached Ligands (continued)

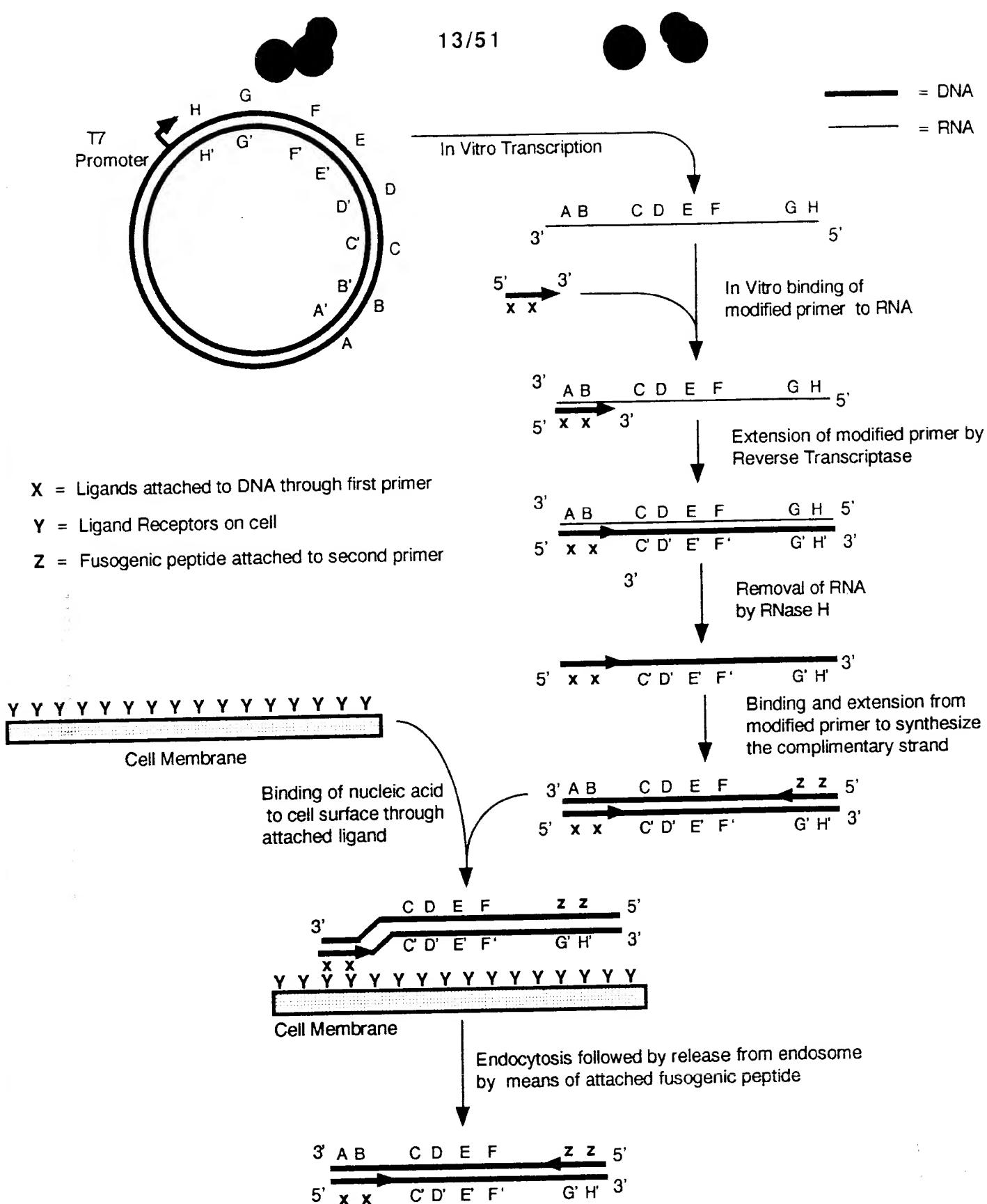


Figure 13

Linear Double-stranded DNA with attached Moieties on each strand

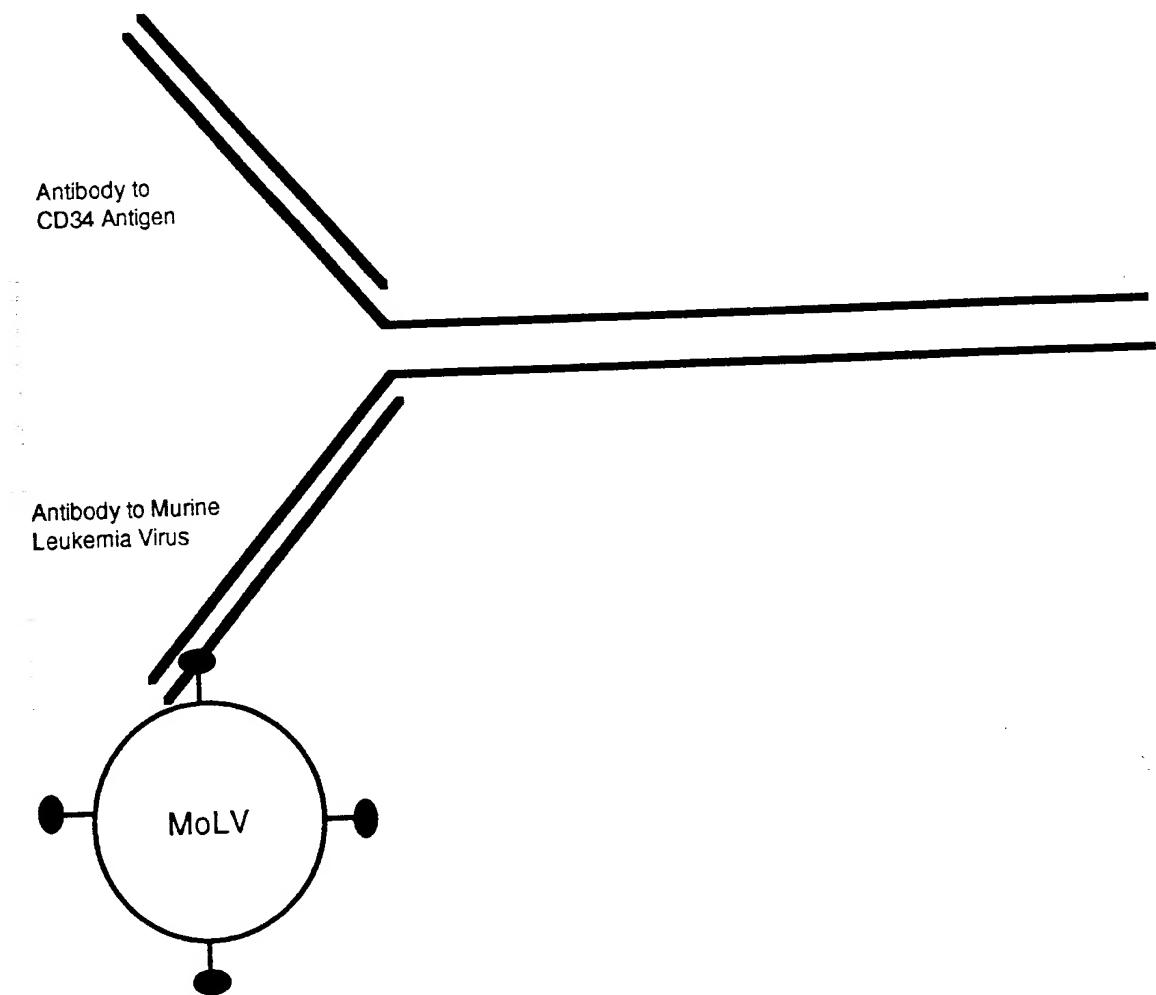


Figure 14
Enhanced Delivery of Retroviral Vector
to Haematopoietic Stem Cell

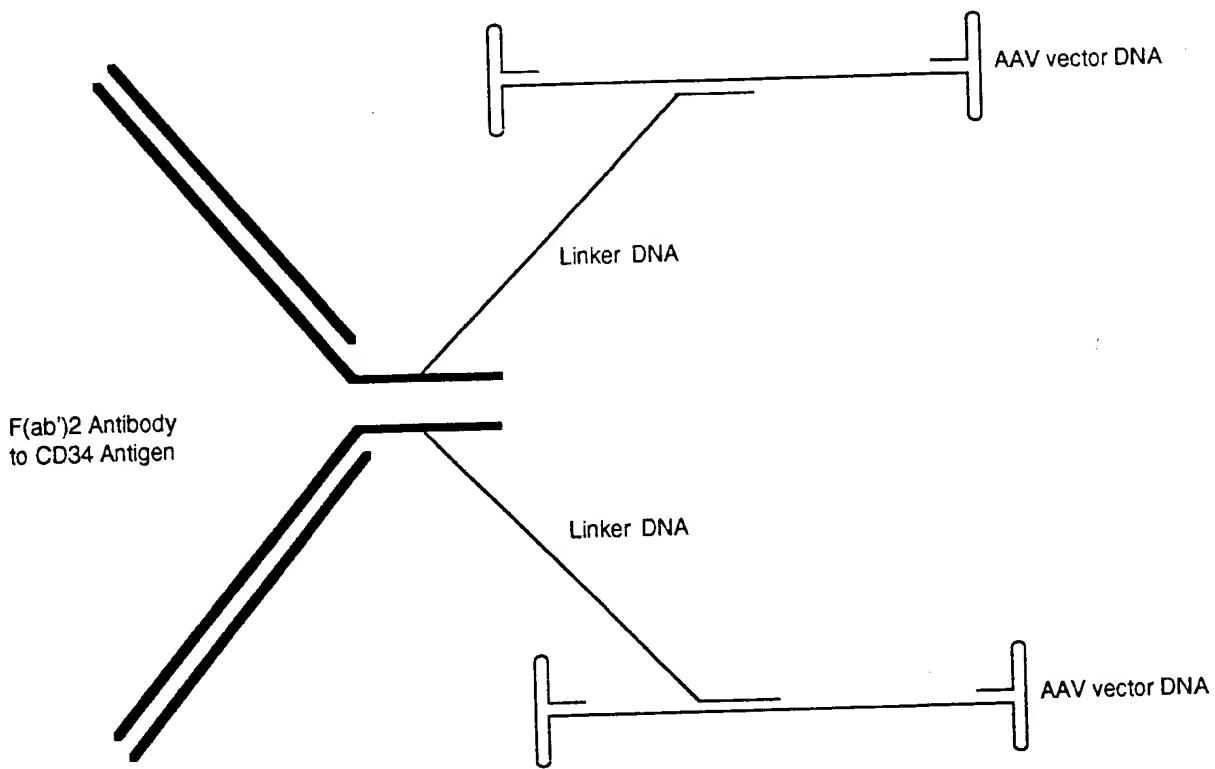
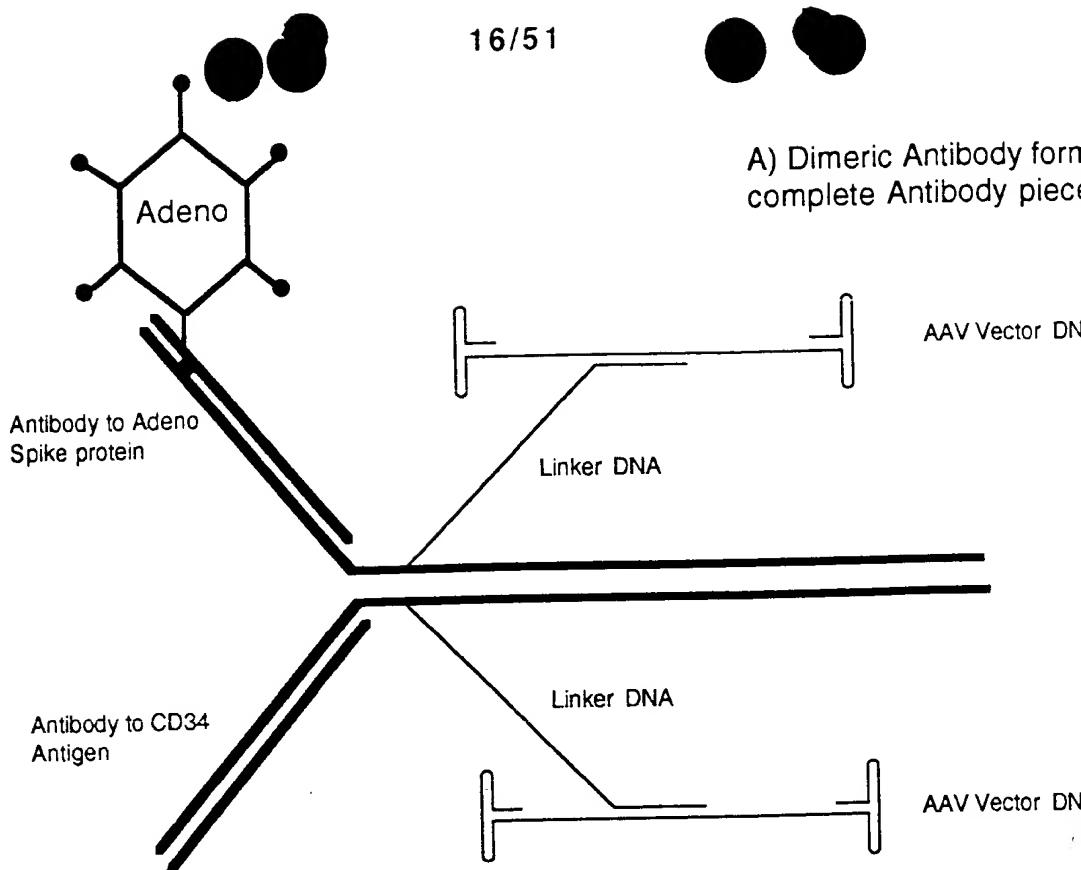


Figure 15
Enhanced Delivery of Vector
DNA to Haematopoietic Stem Cell

A) Dimeric Antibody formed from complete Antibody pieces



B) Dimeric Antibody formed from F(ab') fragments

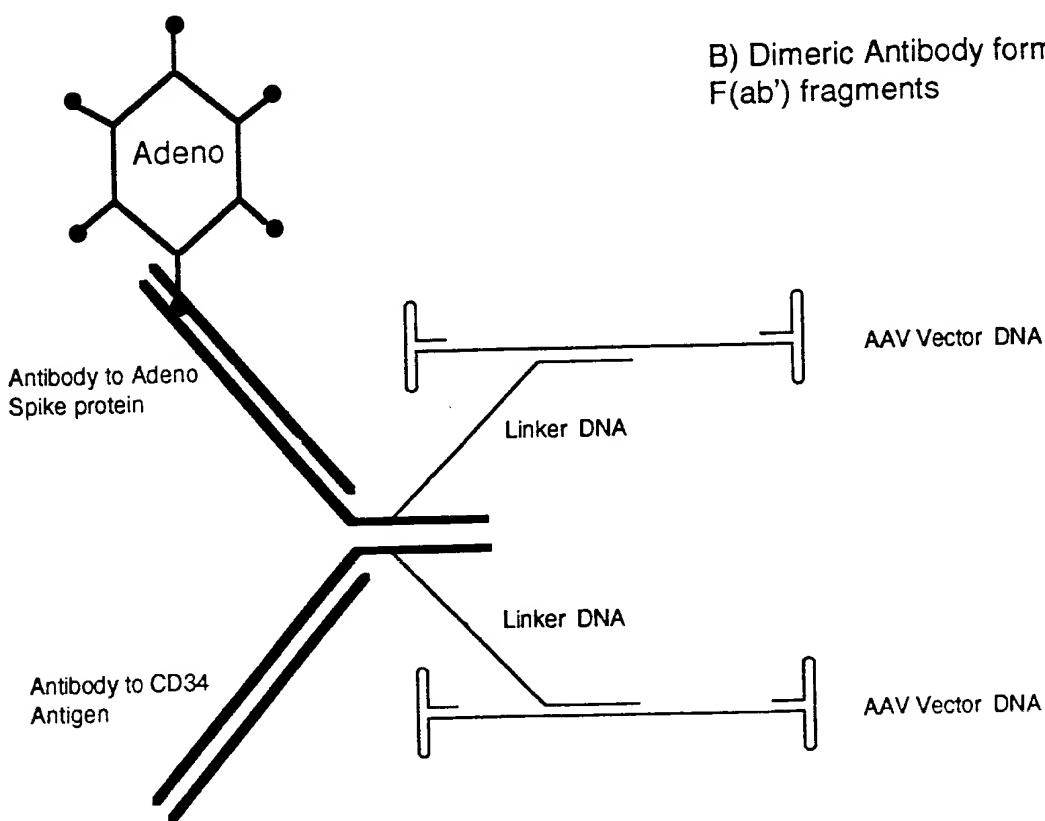


Figure 16
Covalent Attachment of vector DNA to Dimeric Antibody

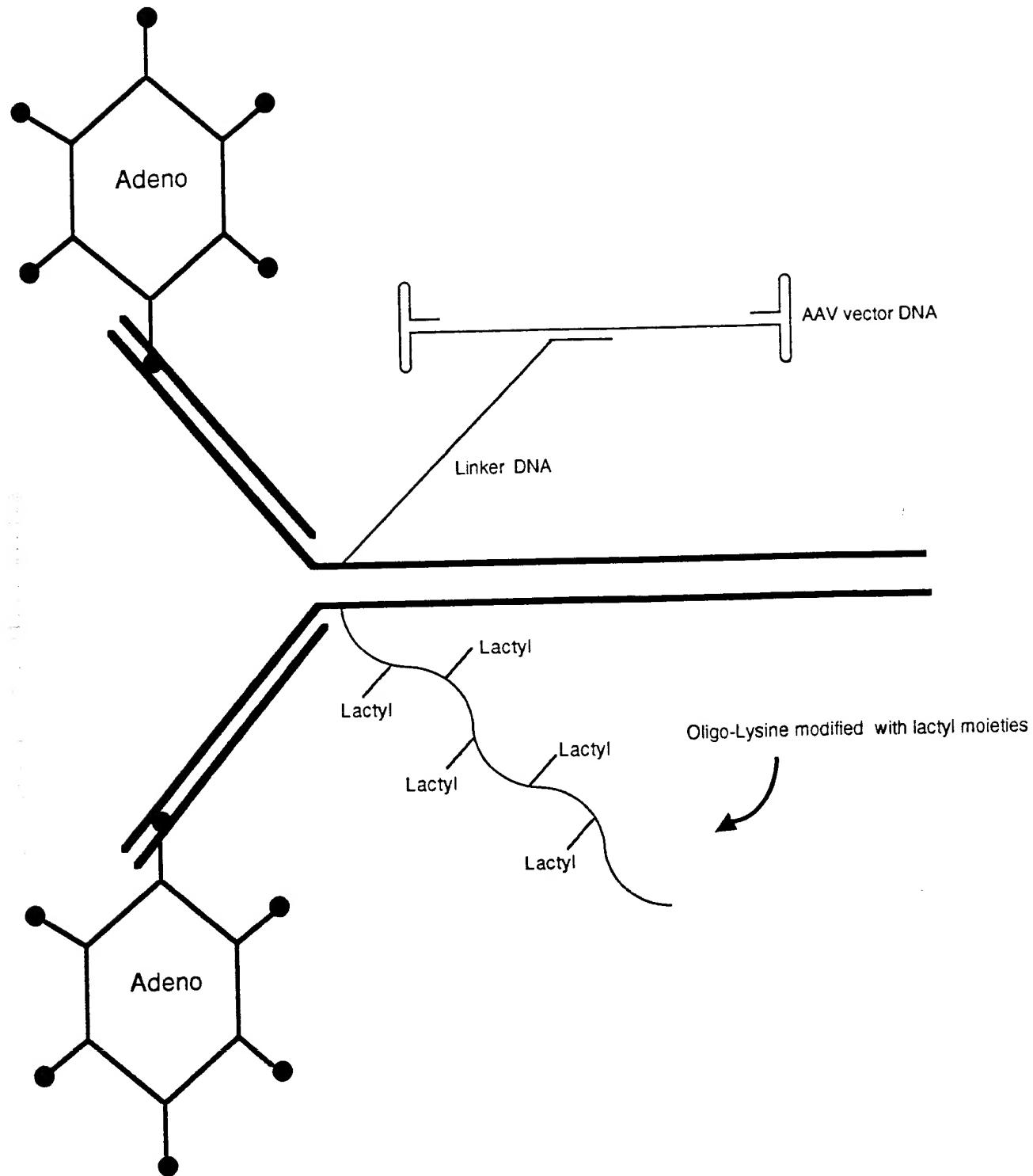


Figure 17
Covalent attachment of Modified DNA
to a Monovalent Antibody

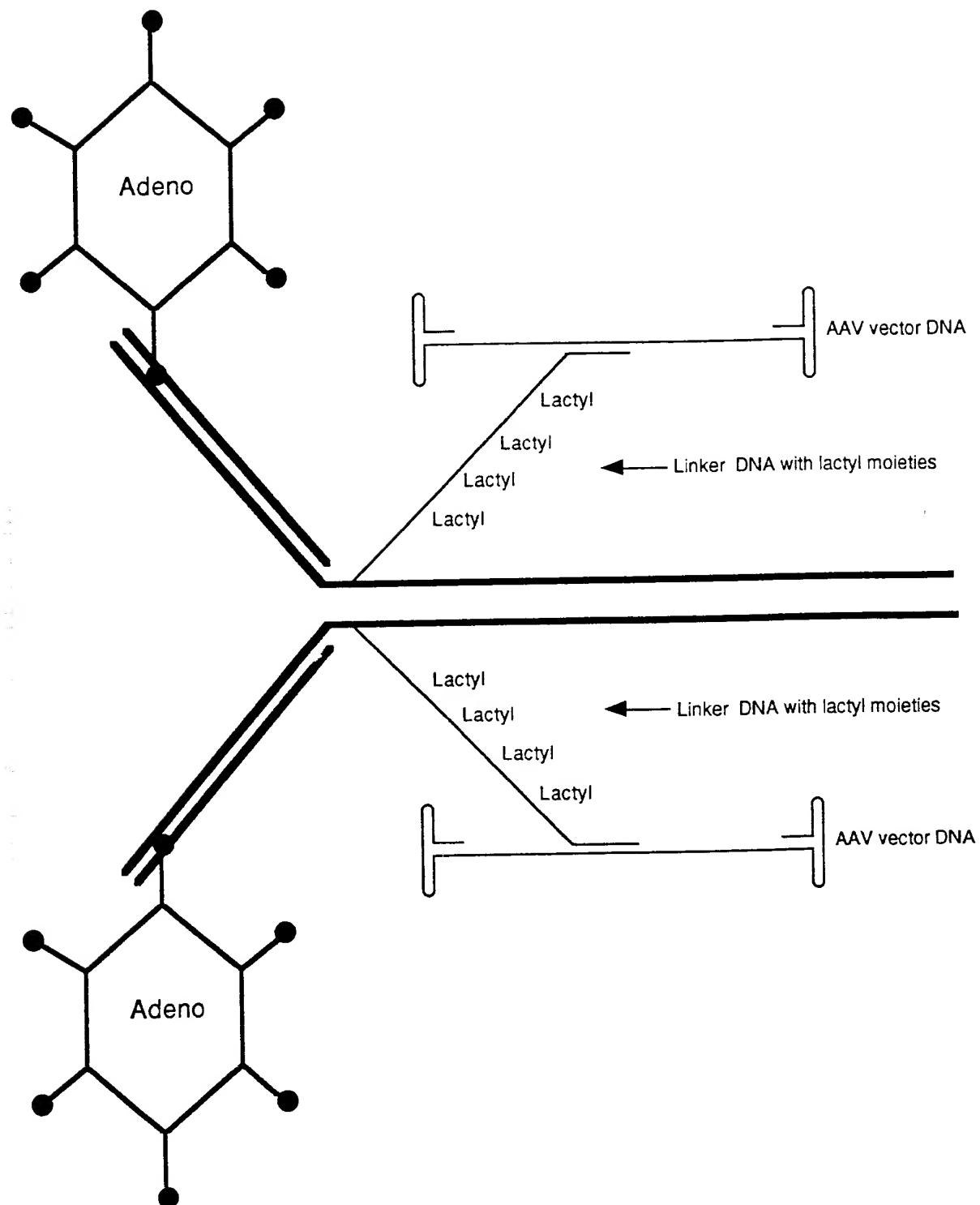
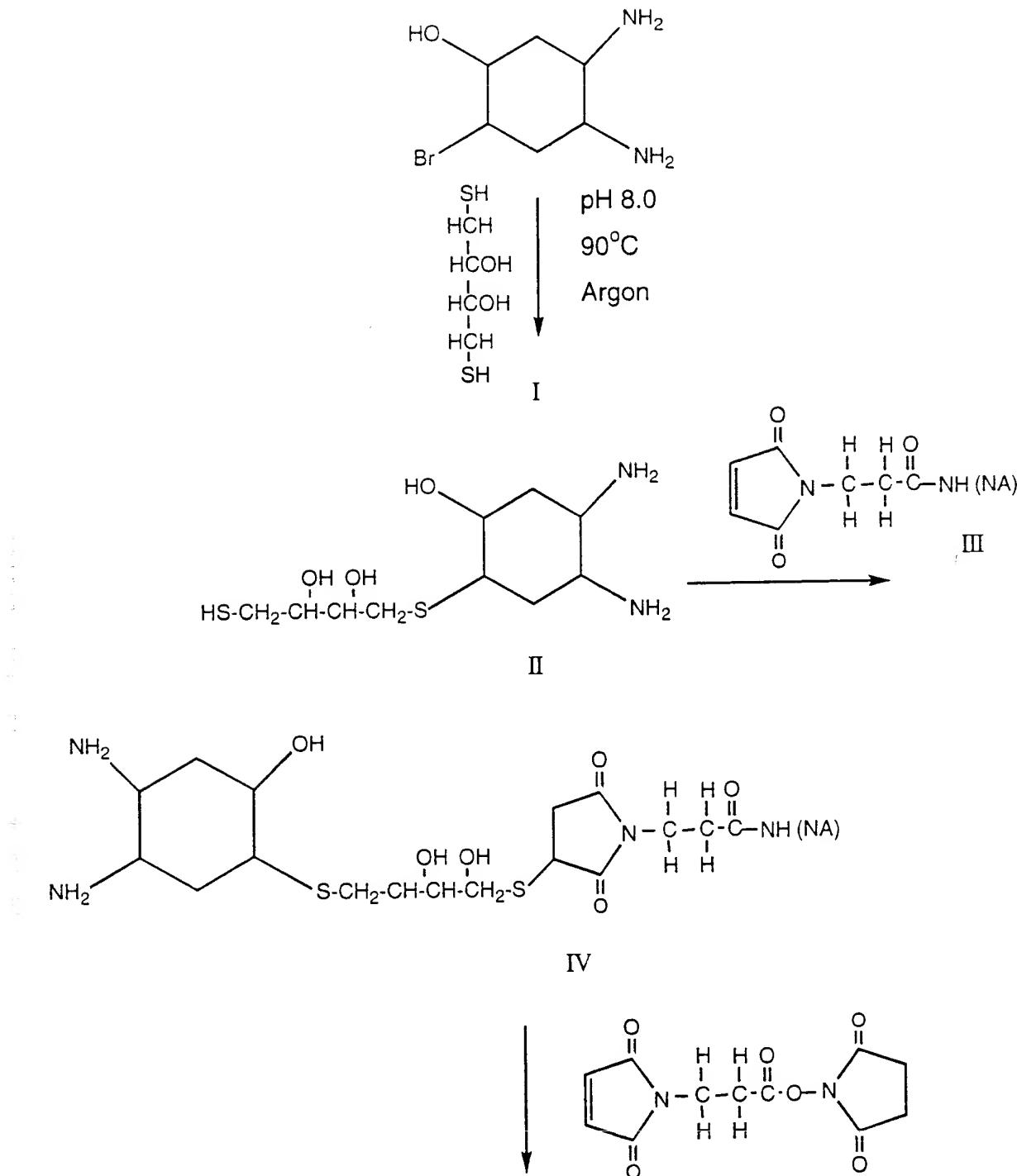


Figure 18
Modified DNA used as a Binder



(continued in Figure 20)

Figure 19
Synthetic Steps for Creation of Antibodies
With Nucleic Acid Moieties Attached

(Continued from Figure 19)

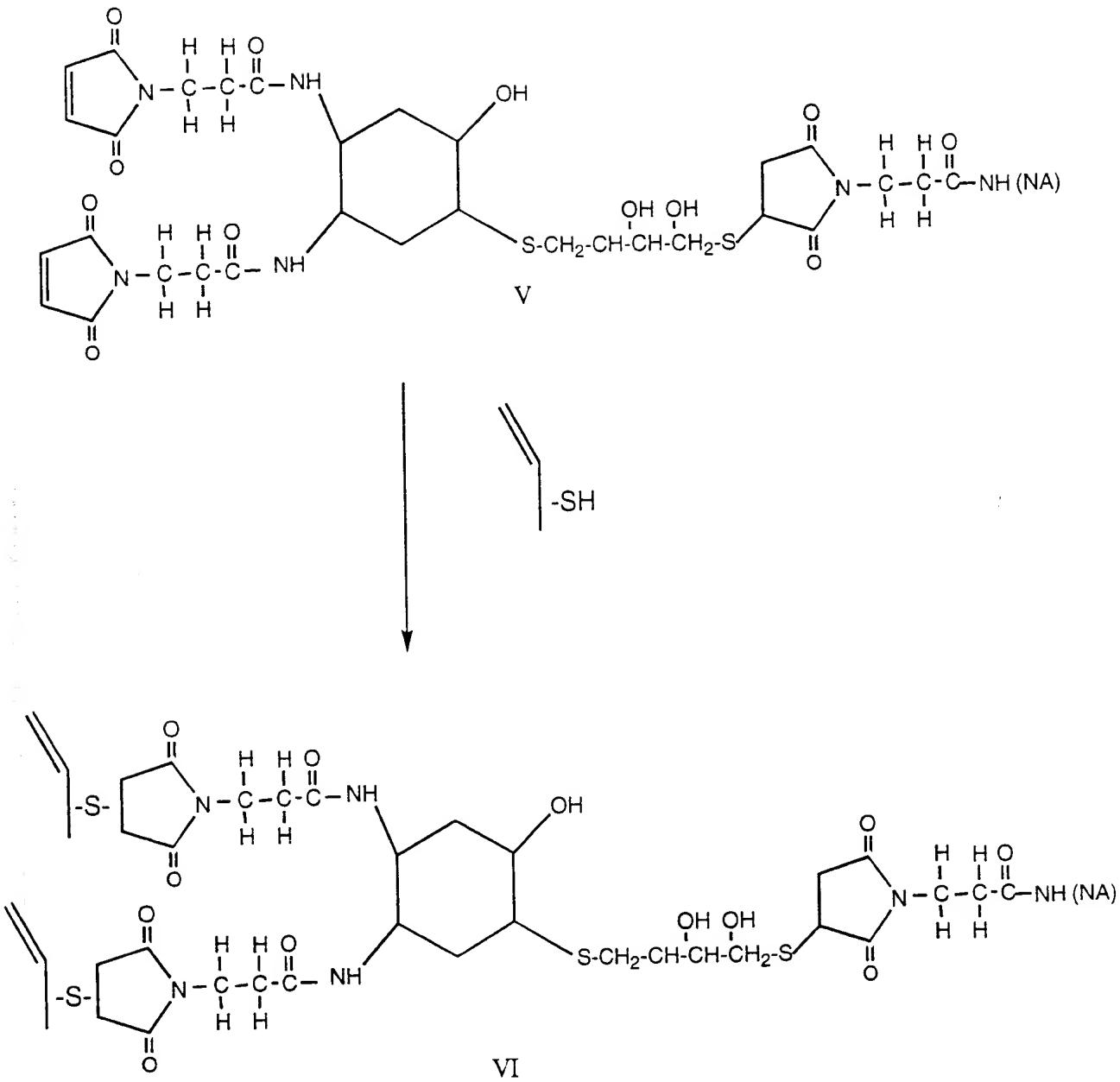
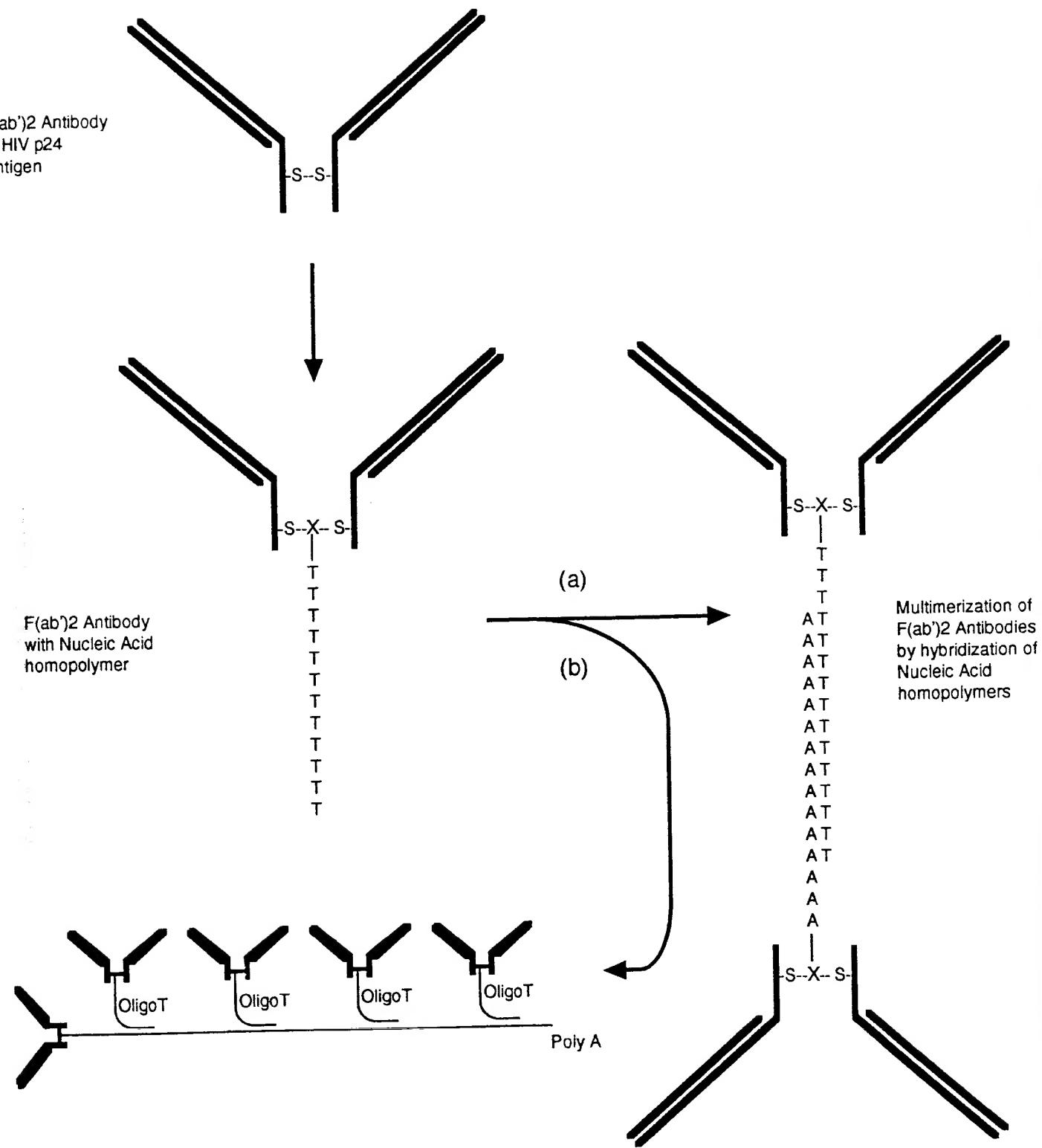


Figure 20
Continuation of Synthetic Steps

**Figure 21**

Enhanced Binding of Antibodies to Antigens by Multimerization

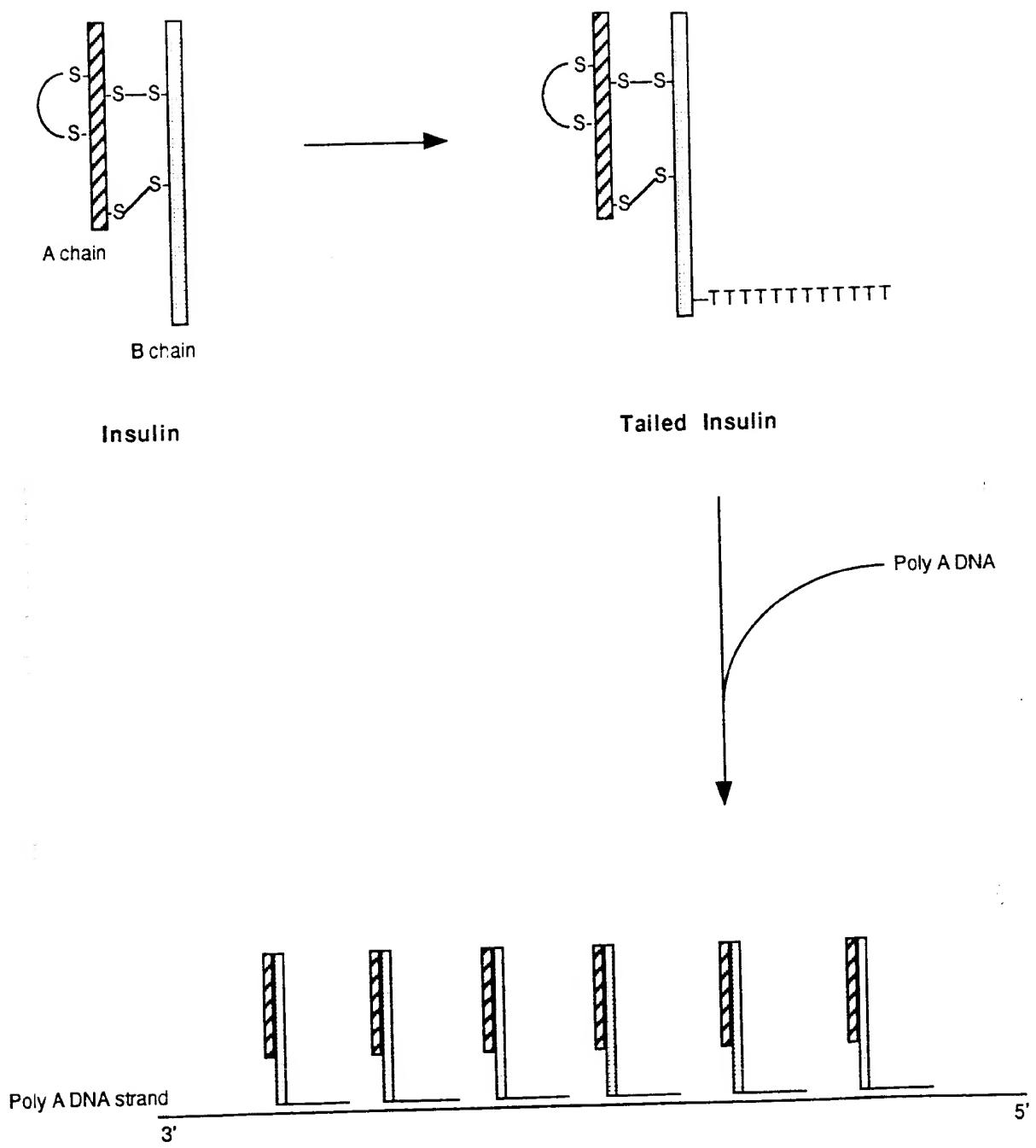


Figure 22
High Affinity Multi-Insulin Soluble Complex

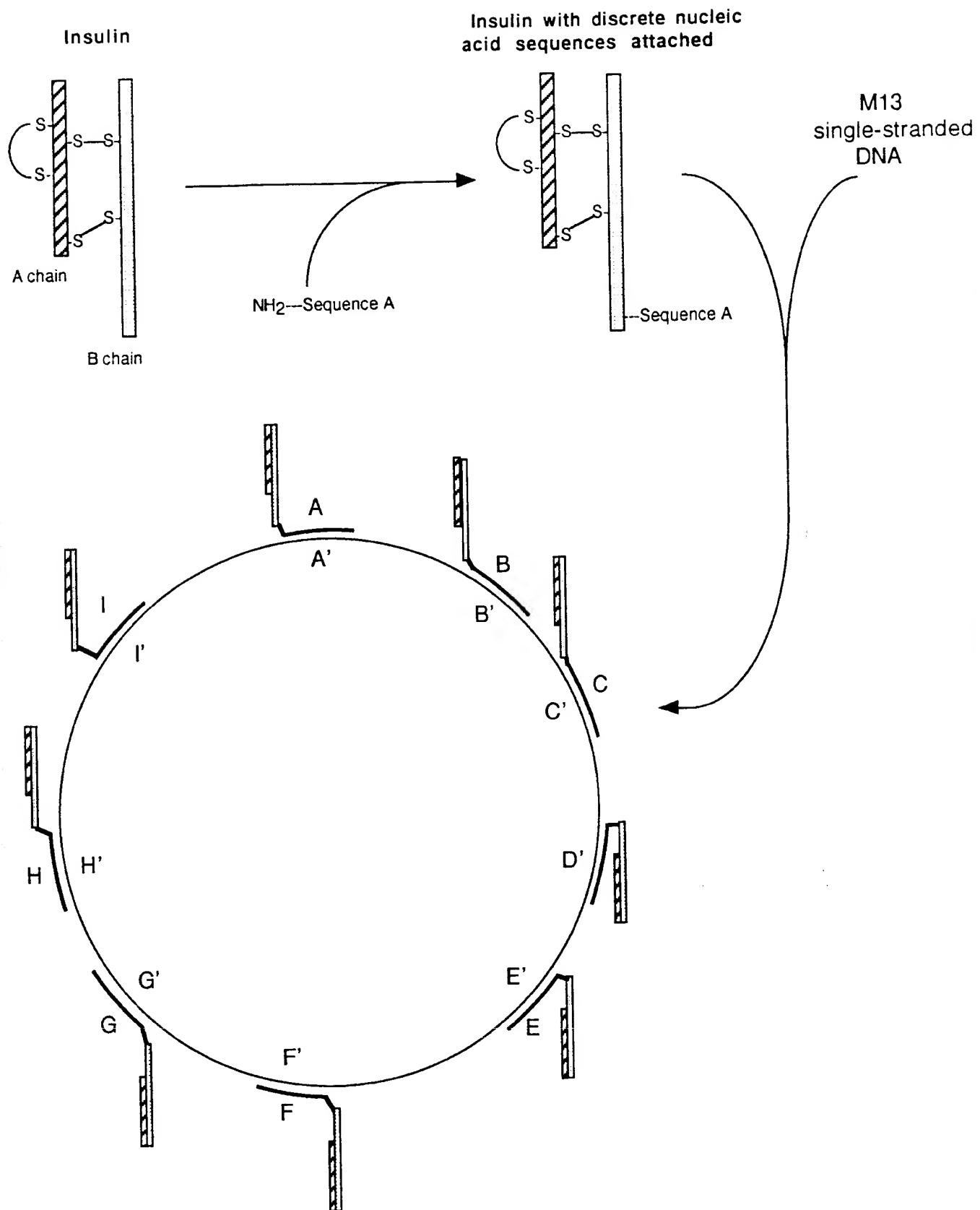


Figure 23

Multimerization of Insulin molecules by hybridization to discrete Sequences

Intron insertion site


(A) - - - **TGCTCTCTAAGGGTCTACTC** - - -
 - - - **ACGAGAGATTCCCAGATGAG** - - -

T7 RNA Polymerase Sequence

SV40 Intron Sequence

(C) **TGCTCTCTAAGGTAAATAT** - - - - - **TGTATTTAGGGTCTACTC**
ACGAGAGATTCCATTATA - - - - - **ACATAAAATCCAGATGAG**

Insertion of SV40 Intron into polymerase coding sequence

mRNA transcript containing intron

(E) -----UGCUCUCUAAGGGUCUACUC-----

mRNA transcript after splicing has normal T7 Sequence

Figure 24

Fusion of Intron into T7 RNA Polymerase Coding Sequence

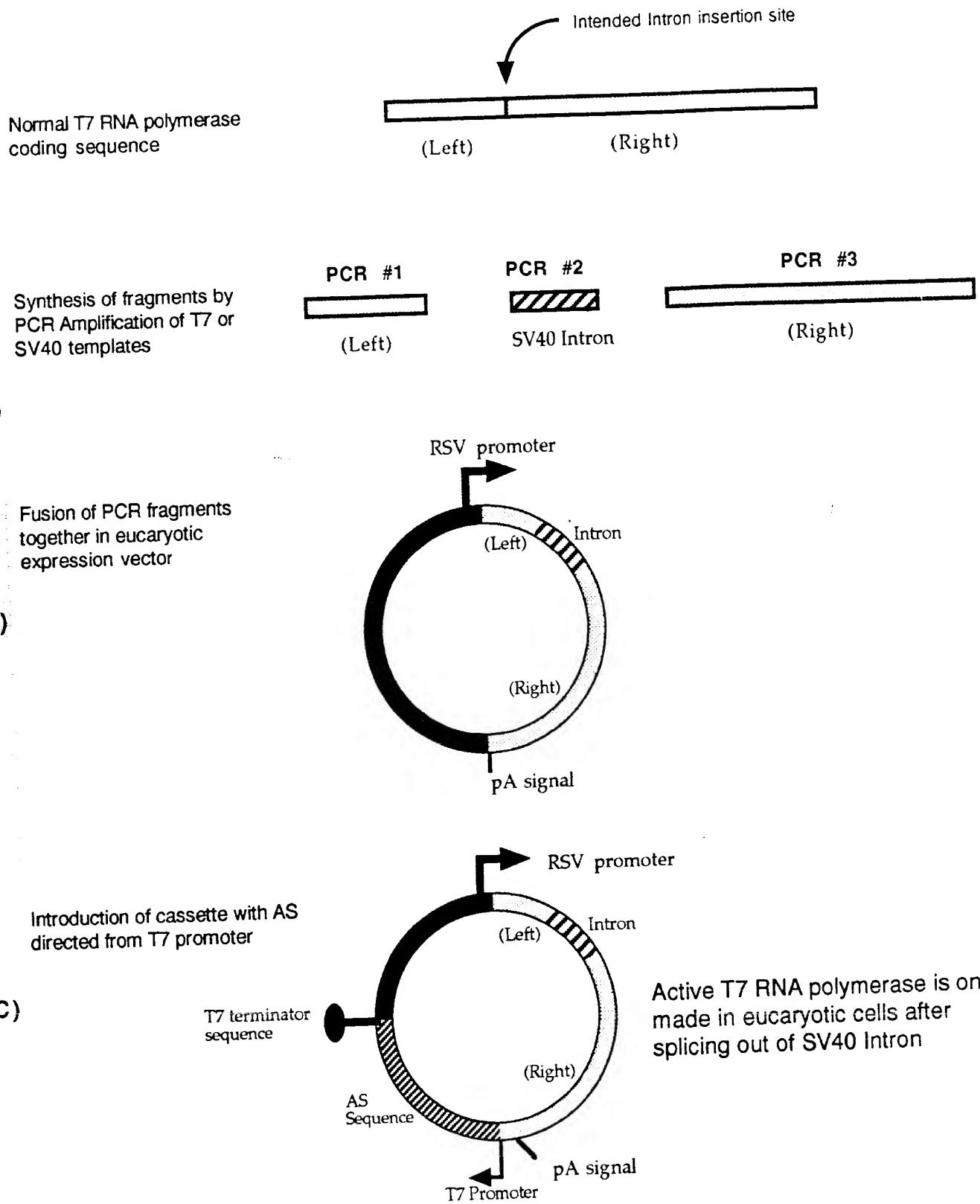
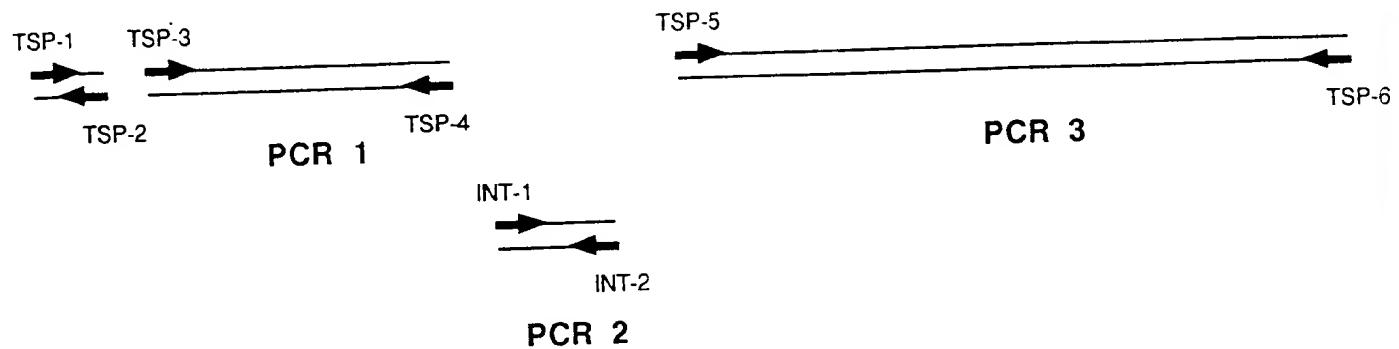


Figure 25
Construction of T7 Expression Vector

A) Synthesis of pieces



B) Oligomers used for synthesis

TSP-1	GGA ATT CGT CTC GAG CTC TGA TCA CCA CCA TGG ACA CGA TTA ACA TCG C
TSP-2	GAC TAG TTG GTC TCG TCT CTT TTT TGG AGG AGT GTC GTT CTT AGC GAT GTT AAT C
TSP-3	GGA ATT CGT CTC GGA GAA AGG TAA AAT TCT CTG ACA TCG AAC TGG C
TSP-4	GAC TAG TGG TCT CCC CTT AGA GAG CAT GTC AGC
TSP-5	GGA ATT CGG TCT CGG GTC TAC TCG GTG GCG AGG
TSP-6	GAC TAG TCG TTA CGC GAA CGC AAA GTC
INT-1	GGA ATT CGT CTC TAA GGT AAA TAT AAA ATT TTT AAG
INT-2	GAC TAG TCG TCT CTG ACC CTA AAA TAC ACA AAC AAT TAG A

Figure 26

Synthesis of Pieces for Construction of
T7 RNA Polymerase with Intron

Formation of Nuclear Localisation Signal by Fusion of TSP1/TSP2 Product to Clone with PCR #1 product

Annealing of TSP1 with TSP2

TSP1

5' GG AAT TCG TCT CAA GCT CTA ATC ACC ACC ATG GAC ACG ATT AAC ATC GC 3',
 3' CC TAA TCG TAG CGA TTC TGA CGA TGA TAA TCG TGC TAA TCG TAC TGG TGA GCA GGT TTT TTC TCT GCT CTT GAT CAG 5'

TSP2

5' AG AAT TCG TCT CGA GCT CTA ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAC GAC ACT CCT CCA AAA AAG AGA CGA GAC CAA CTA GTC 3'
 3' C TAA TCG TAG CGA TTC TGA CGA TGA TAA GCA TGC TGG CTG TGA GGA GGT TTT TTC TCT GCT CTT GAT CAG 5'

Bsa I

Extension of TSP1/TSP2 by polymerase

Figure 27

Annealing of TSP1 with TSP2

5' GG AAT TCG TCT CAA GCT CTA ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAC GAC ACT CCT CCA AAA AAG AGA CGA GAC CAA CTA GTC 3',
 3' CC TAA AGC AGA GCT CGA GAC GTC TAA TCG TAC TGG TGA CGA TTC TGA CGA TGA GGA GGT TTT TTC TCT GCT CTT GAC CAG 5'

Extension of TSP1/TSP2 by polymerase

5' GG AAT TCG TCT CAA GCT CTA ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAC GAC ACT CCT CCA AAA AAG AGA CGA GAC CAA CTA GTC 3',
 3' CC TAA AGC AGA GCT CGA GAC GTC TAA TCG TAC TGG TGA CGA TTC TGA CGA TGA GGA GGT TTT TTC TCT GCT CTT GAC CAG 5'

Digestion of PCR #1 clone (pL-1) with BsmB I

5' GGA ATT CGT CRC G Bsm B1 GAG AAG GAA AAA TTC TCT GAC ATC GAA CTC GC-----
 CCT TAA GCA GAG CCTCTT TTC CAT TTT AAG AGA CGA TGT TGG CTT GAC CG-----

Ligation of Bsa I digested TS1/TS2 product to BsmB I digested PCR#1 clone

5' GG AAT TCG TCT CGA GCT CTA ATC ACC ACC ATG GAC ACT ATT AAC ATC GCT AAC GAC ACT CCT CCA AAA AAG AGA CGA GAC CAA CTA GTC 3',
 3' CC TAA AGC AGA GCT CGA GAC GTC TAA TCG TAC TGG TGA CGA TTC TGA CGA TGA GGA GGT TTT TTC TCT GCT CTT GAT TTT AAG

TCT GAC ATC GAA CTG GC-----
 AGA CTG TAG CTT GAC CG-----

Comparison of the 5' ends of the Nucleotide Sequences of Wild Type
and Modified T7 RNA Polymerase

Wild Type T7 nucleic and amino acid sequence

ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC TTC TCT GAC ATC GAA CTG GC-----
TAC CTG TGC TAA TTG TAG CGA TTC TGT CAG AAG AGA CTTG TAG CTTG GAC CG-----
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Modified T7 nucleic and amino acid sequence
with Nuclear Localisation Signal (NLS) insertion

ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA AAG GAA AAA TTC TCT GAC ATC GAA CTG GC-----
TAC CTG TGC TAA TTG TAG CGA TTC TGT CAG AAG AGA CTTG TAG CTTG GAC CG-----
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure 28

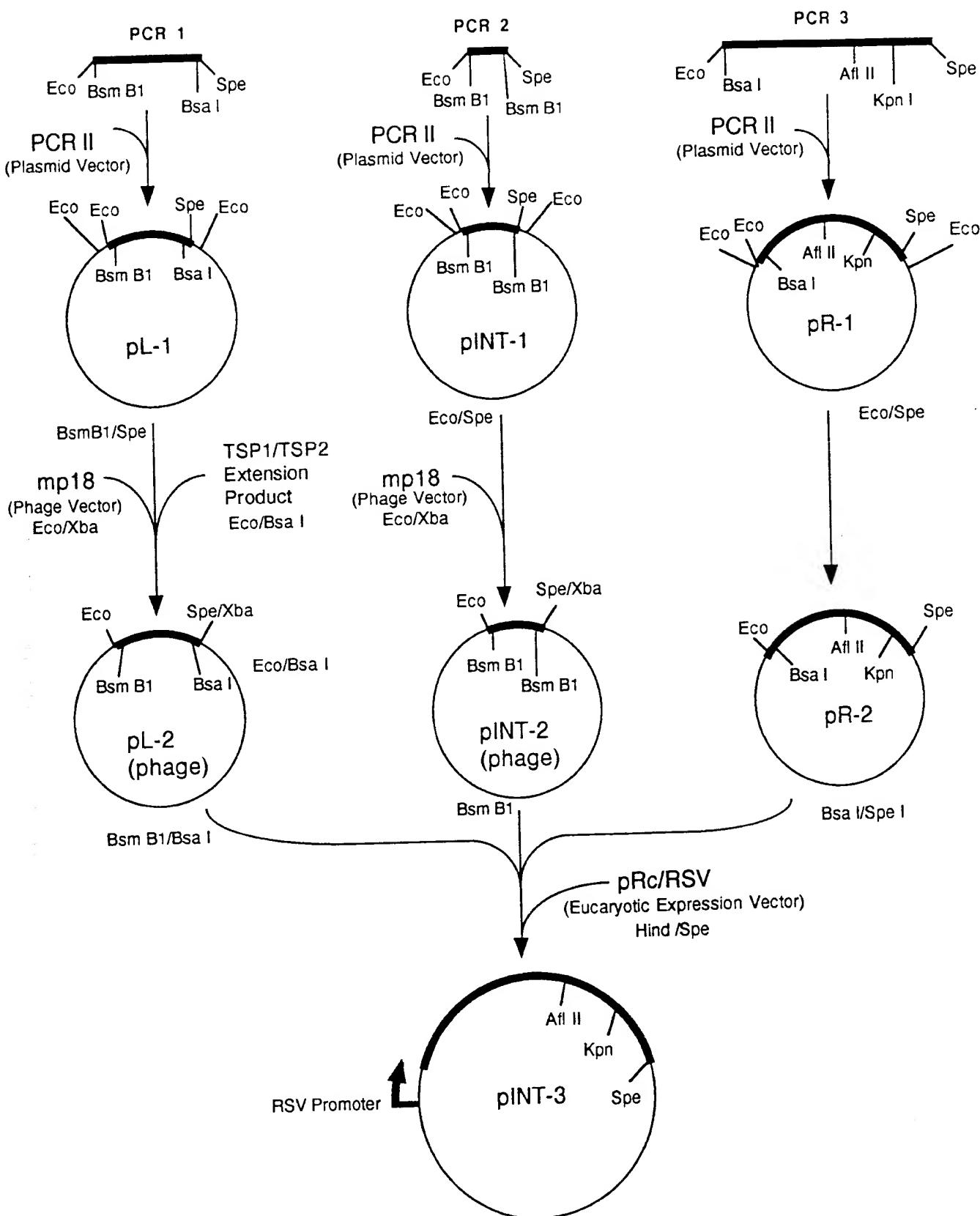


Figure 29
Fusion of PCR Pieces to Construct
T7 RNA Polymerase with an Intron

(A) Oligomers

HTA-1 GAT CAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGCTTA AGC CTC AAG
 HTA-2 GAT CCT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT

 HTB-1 GAT CAC CTT AGG CTC TCC TAT GGC AGG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA G
 HTB-2 GAT CCT TGA GGA GGT CTT CGT CGC TGT CTC CGC TTC CTG CCA TAG GAG AGC CTA AGG T

 HTC-1 GAT CAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC AG
 HTC-2 GAT CCT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT

 TER-1 AAT CTA GAG CTA ACA AAG CCC GAA AGG AAG
 TER-2 TTC TGC AGA TAT AGT TCC TCC TTT CAG C

(B) Cloning of AS and Terminator sequences into vector with T7 Promoter

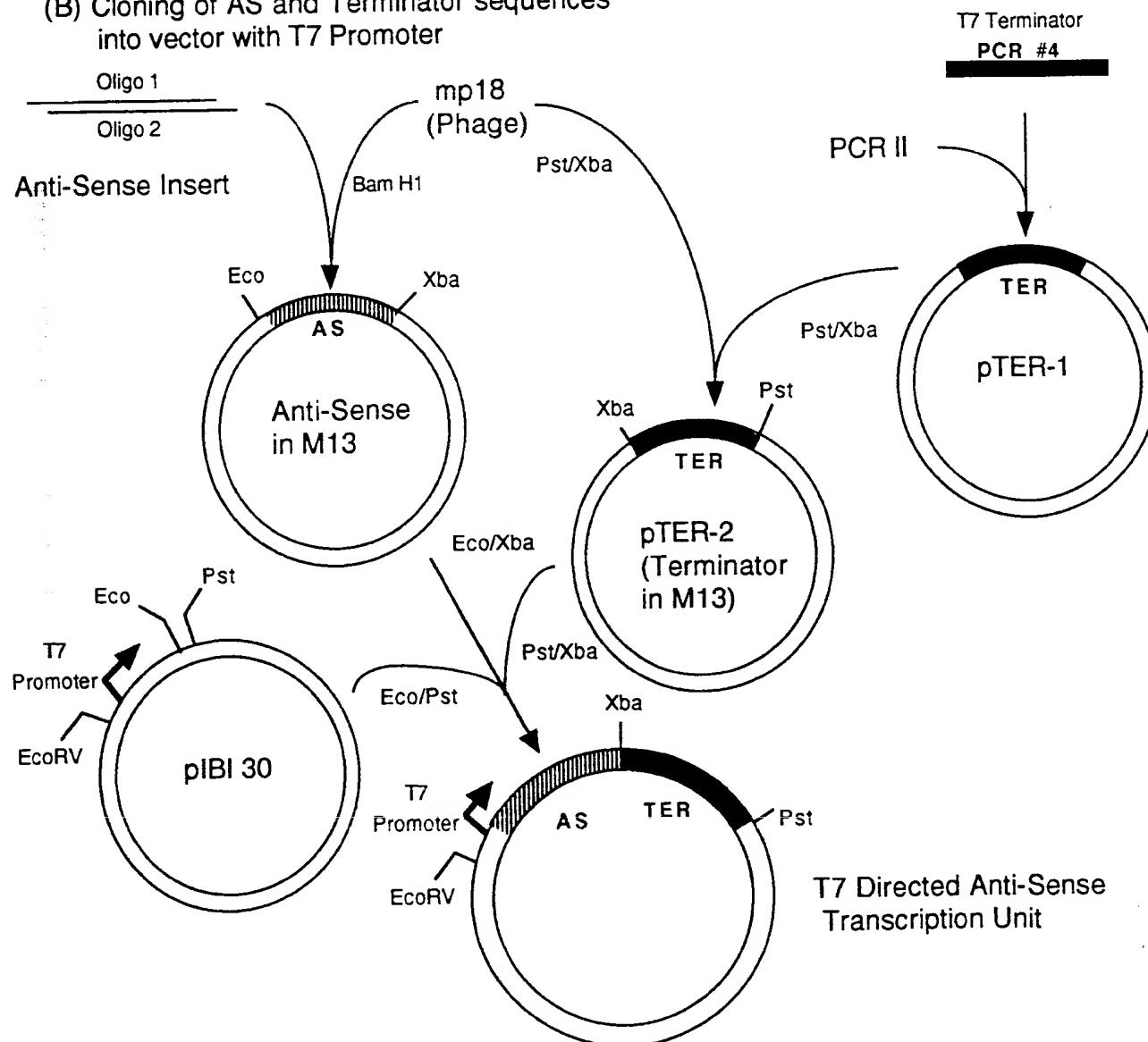


Figure 30
Insertion of Anti-Sense Sequences into
T7 Directed Transcription Units

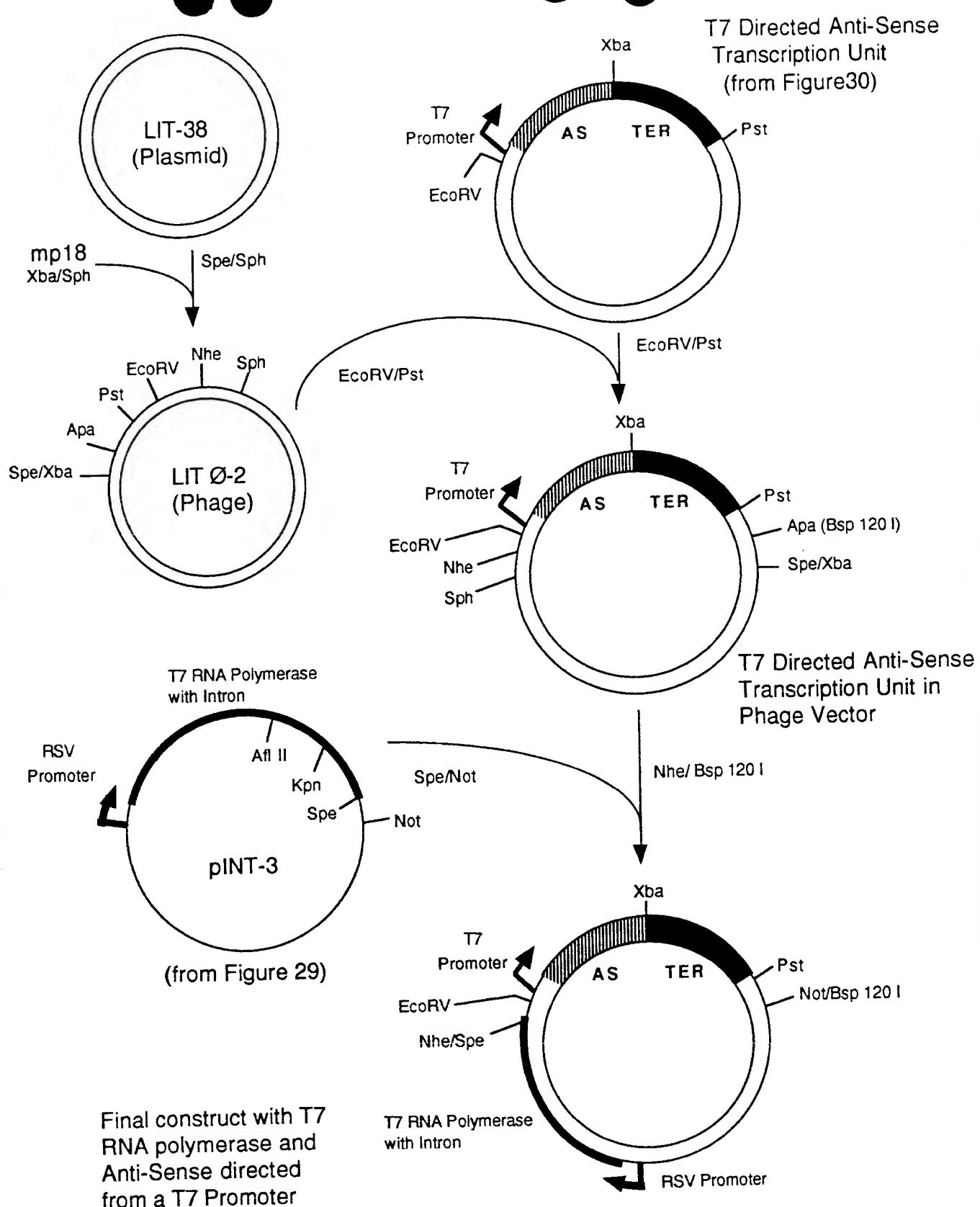


Figure 31
Construct with T7 RNA polymerase and
Anti-Sense directed from a T7 Promoter

A) Oligomers for introduction of T7 signals and polylinker

PL-1

TCG AGC CAT GGC TTA AGG ATC CGT ACG TCC GGA GCT AGC GGG CCC ATC GAT ACT
 AGT TAA ATG CAG ATC T

PL-2

CTA GAG ATC TGC ATT TAA CTA GTA TCG ATG GGC CCG CTA GCT CCG GAC GTA CGG
 ATC CTT AAG CCA TGG C

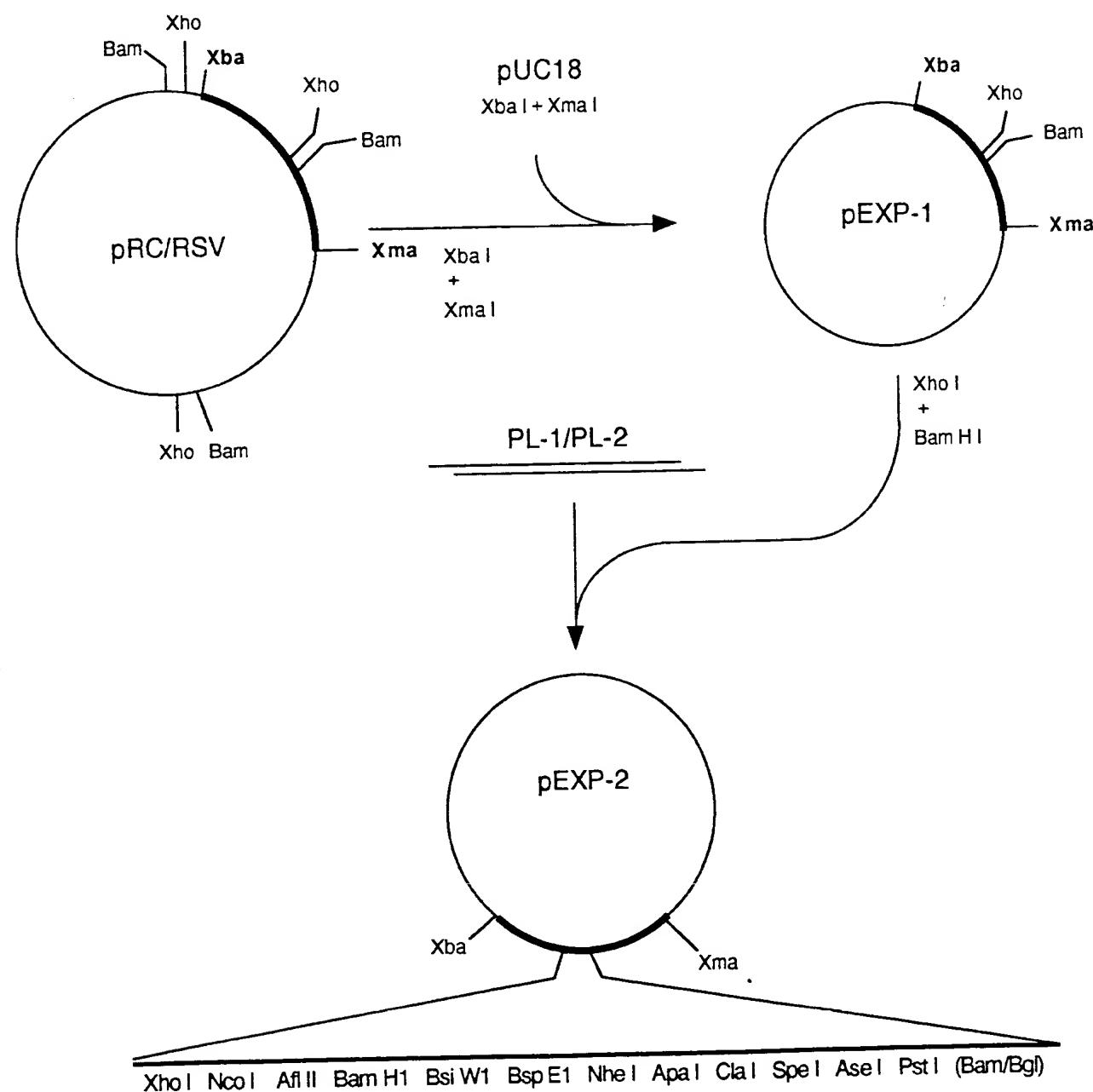


Figure 32

Introduction of Poly-Linker for Creation of Protein Expression Vector

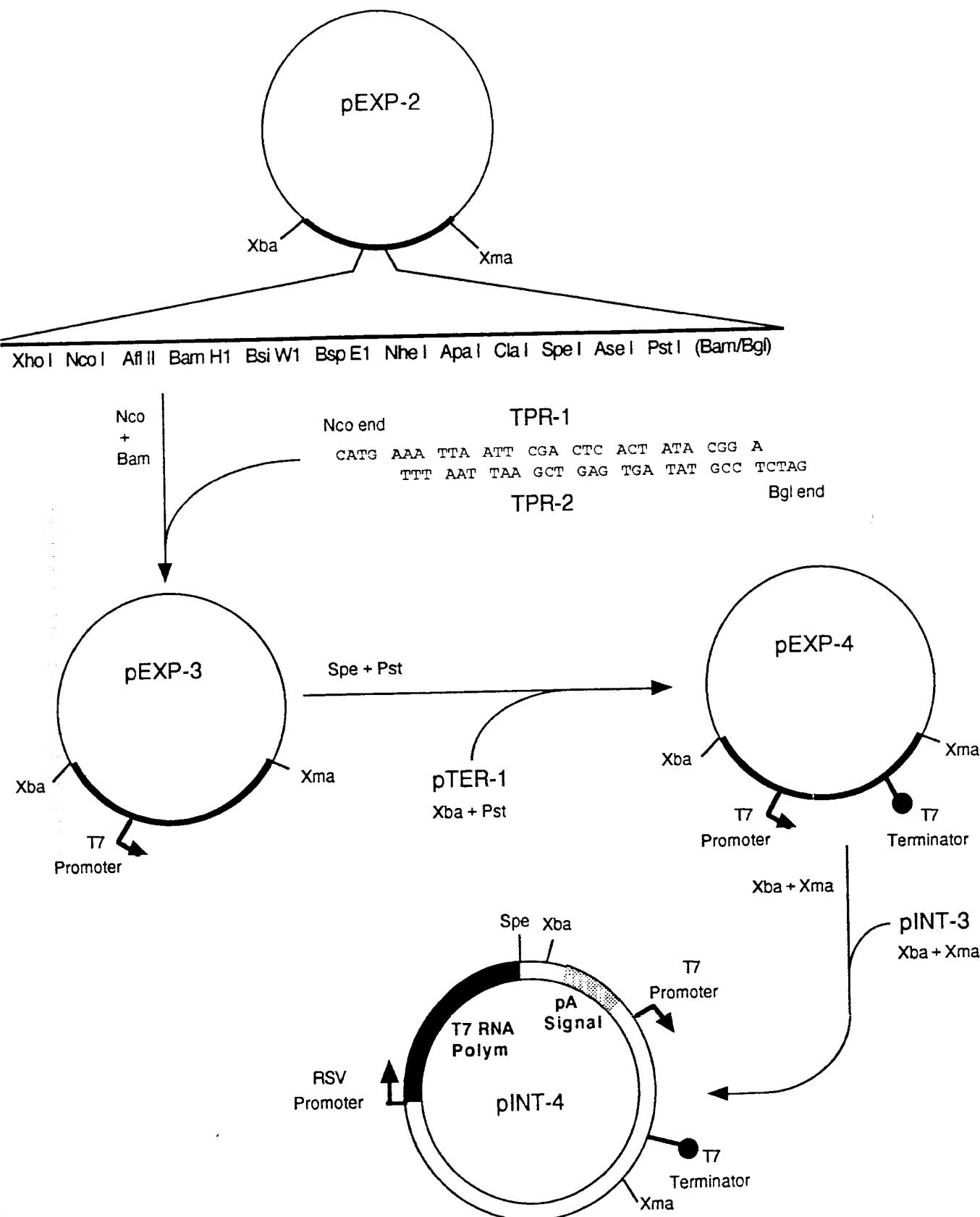


Figure 33

Final steps for construction of Expression Vector

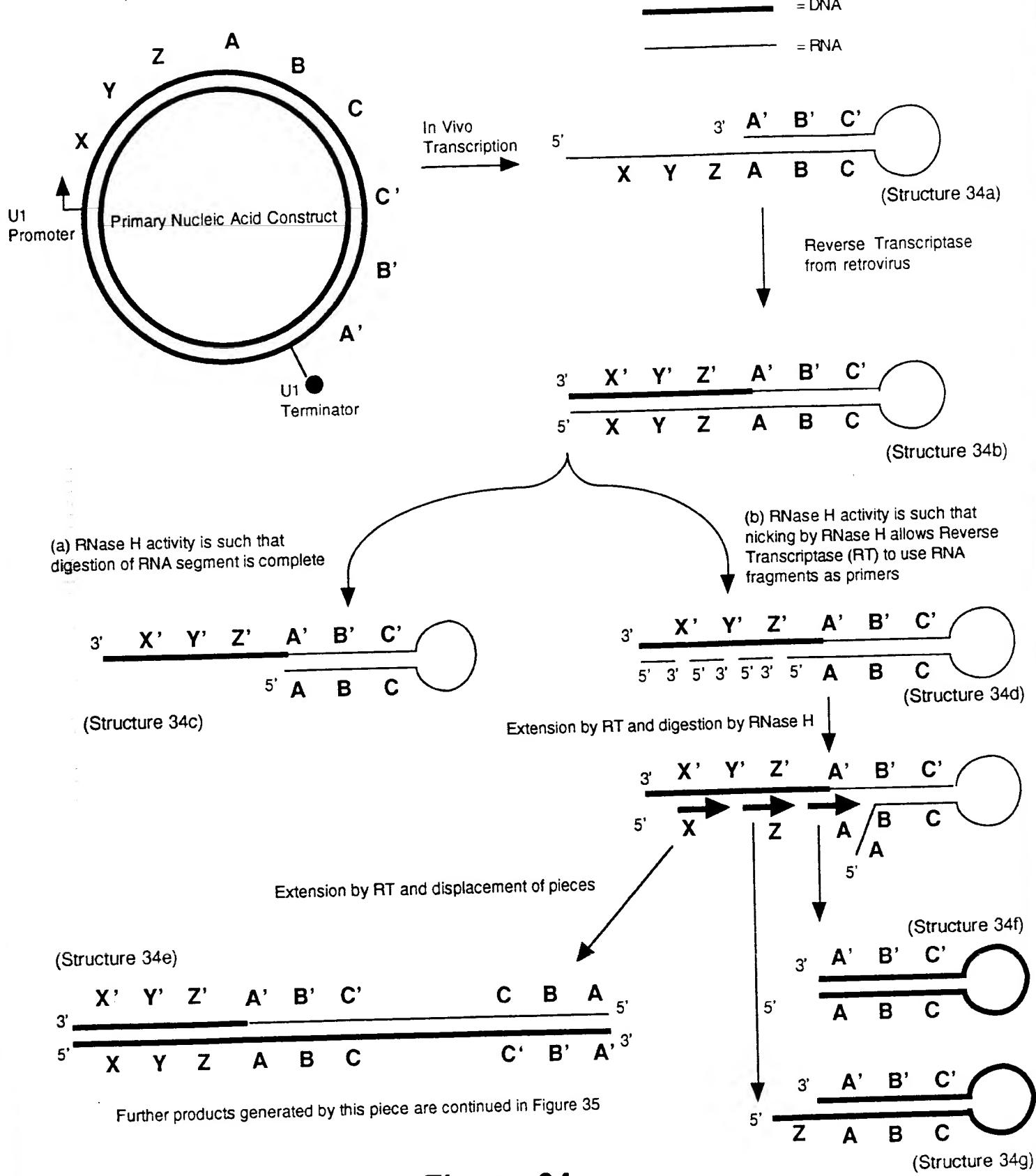
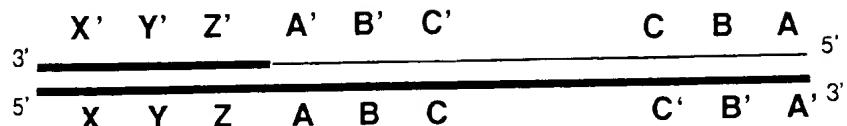


Figure 34

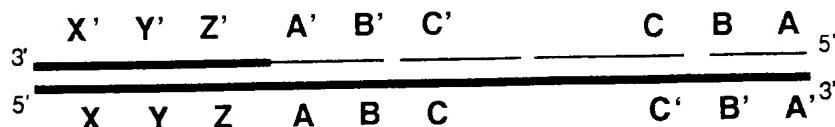
Construct that produces single-stranded Anti-Sense DNA

Continued from Figure 34

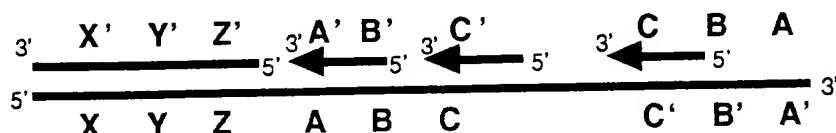
(Structure 34e)



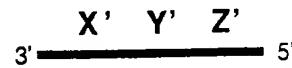
Nicking by RNase H



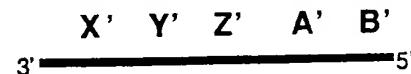
Extension by RT and digestion by RNase H



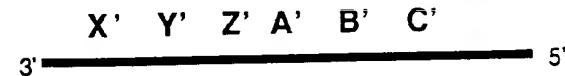
(Structure 35h)



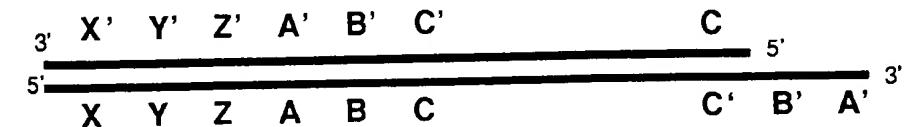
(Structure 35i)



(Structure 35j)



(Structure 35k)



Extension by RT and displacement generates Single-Stranded DNA and a mostly Double-stranded DNA molecule

Figure 35
Continuation of Process from Figure 34

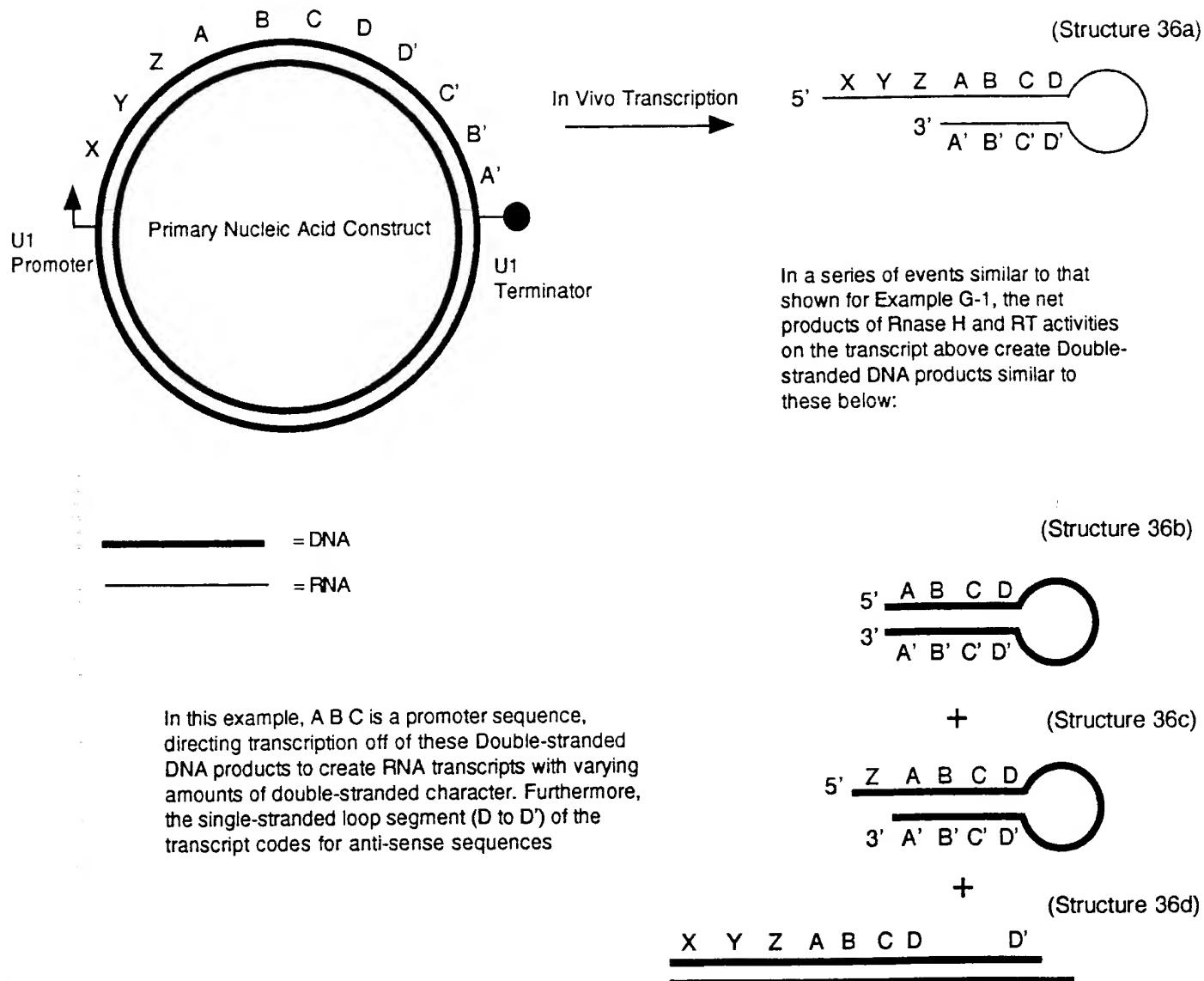
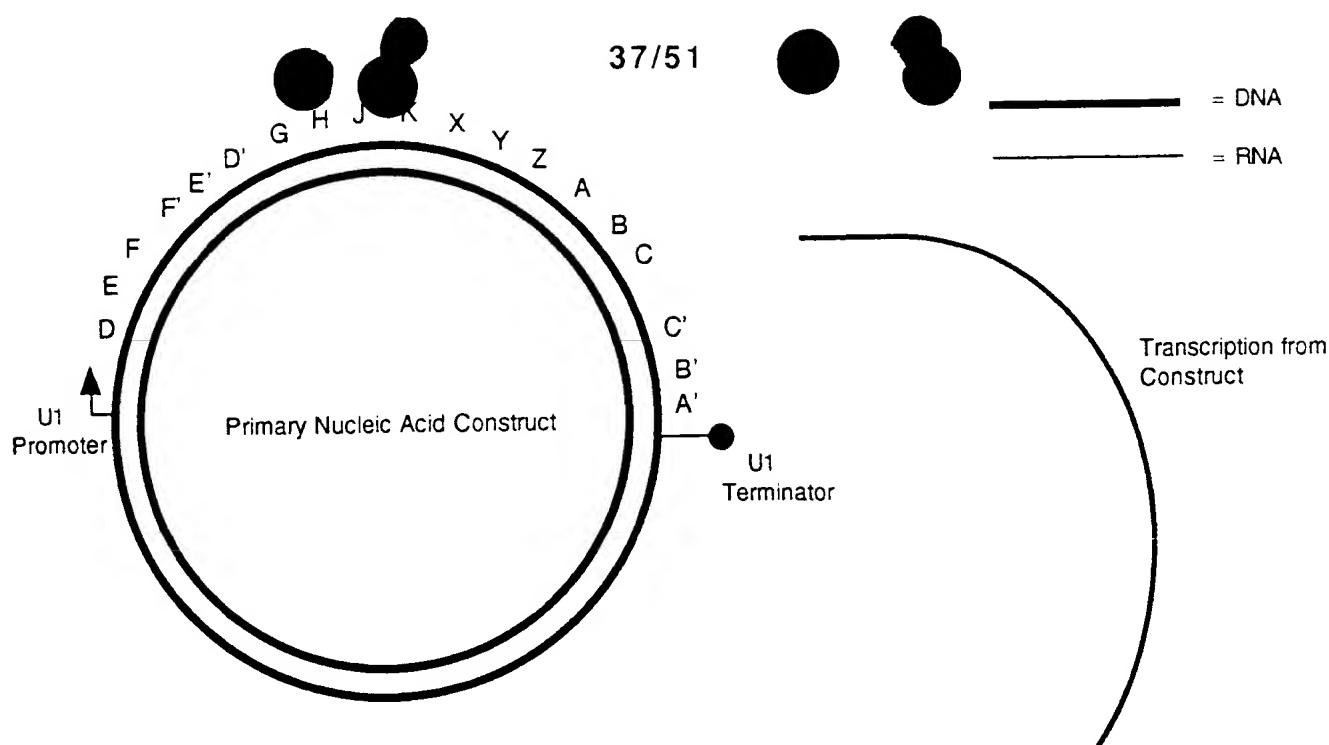


Figure 36

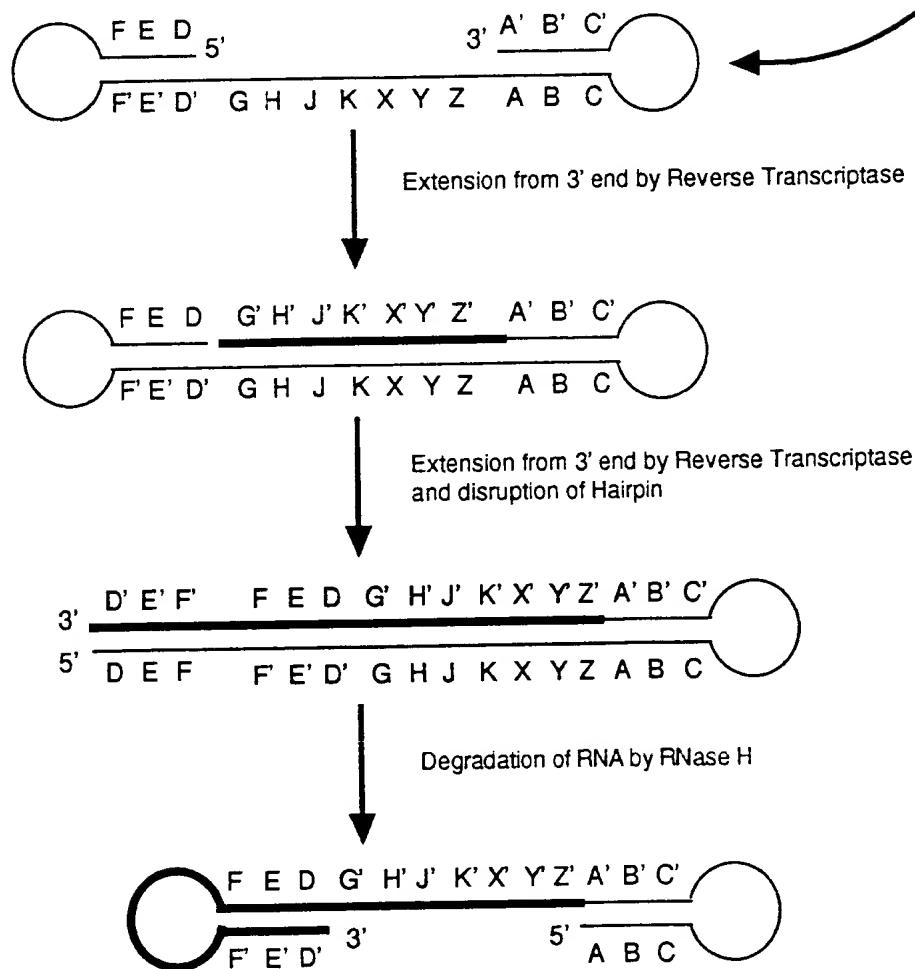
Construct that produces RNA that is Reverse Transcribed to create Secondary DNA Constructs capable of directing transcription

= DNA

= RNA



(Structure 37a)



(Continued in Figure 38)

Figure 37
Construct which Propagates a Double Hairpin Production Center

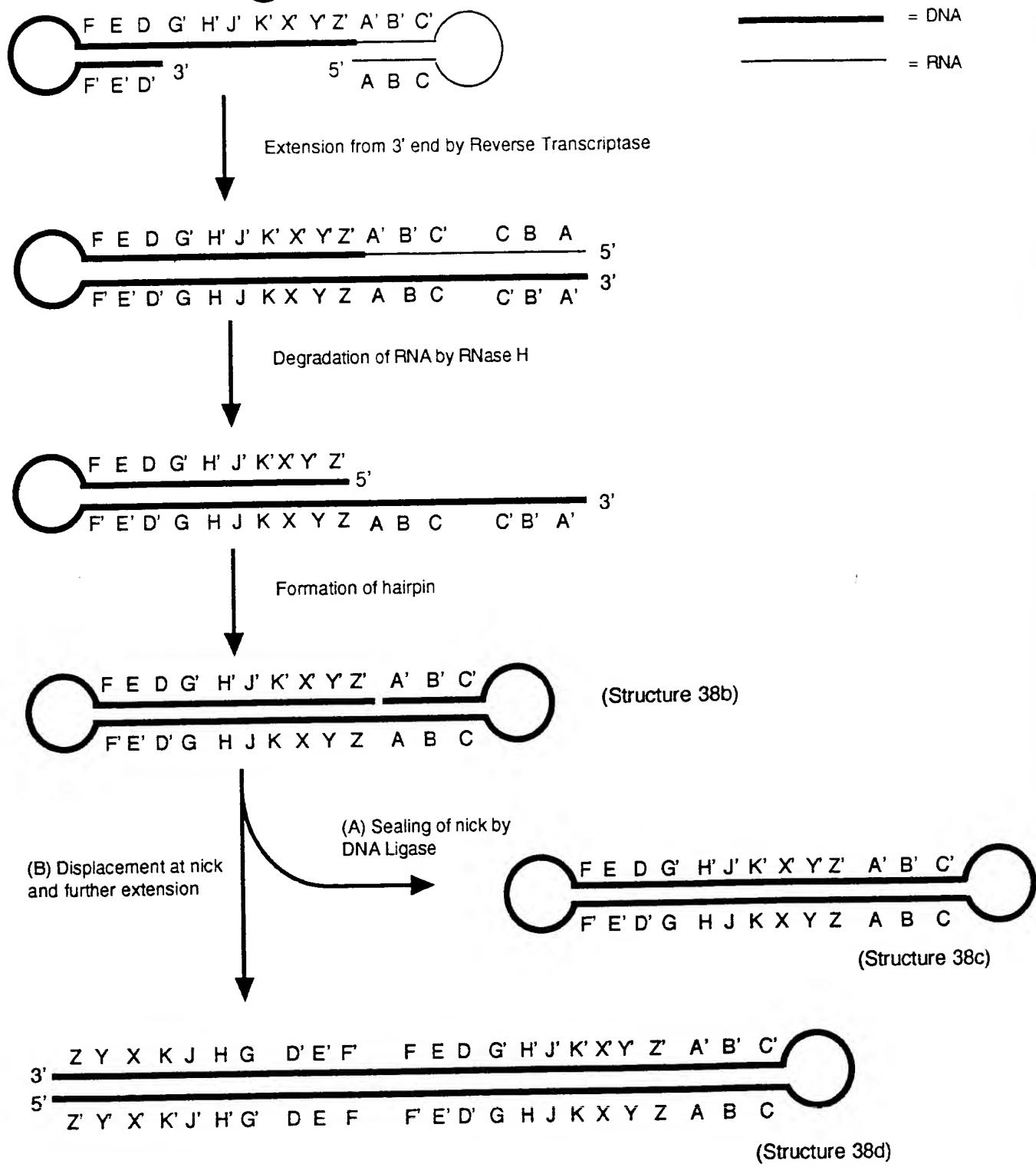


Figure 38
 Continuation of process from Figure 37

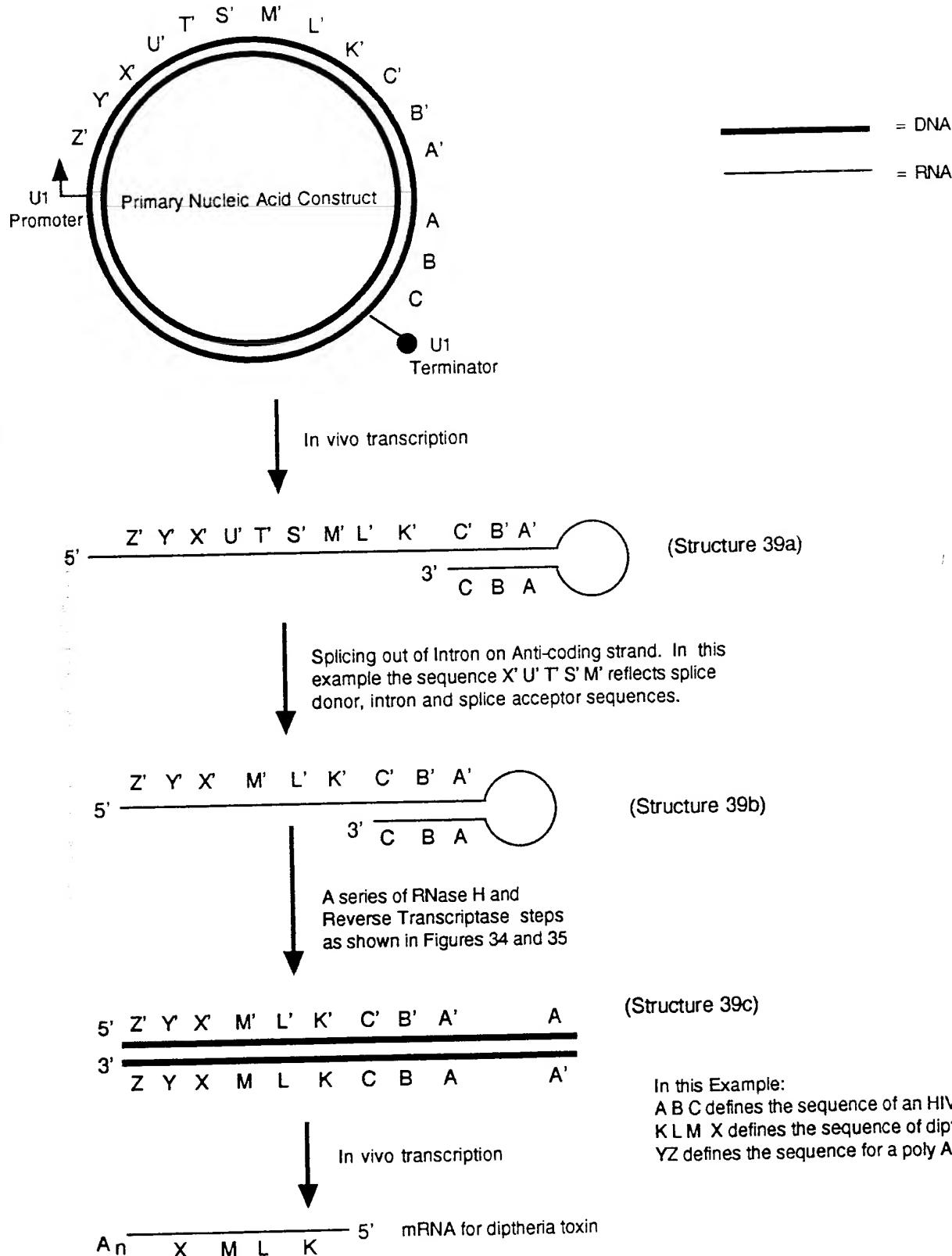


Figure 39

Construct which propagates a Production Center capable of Inducible Suicide

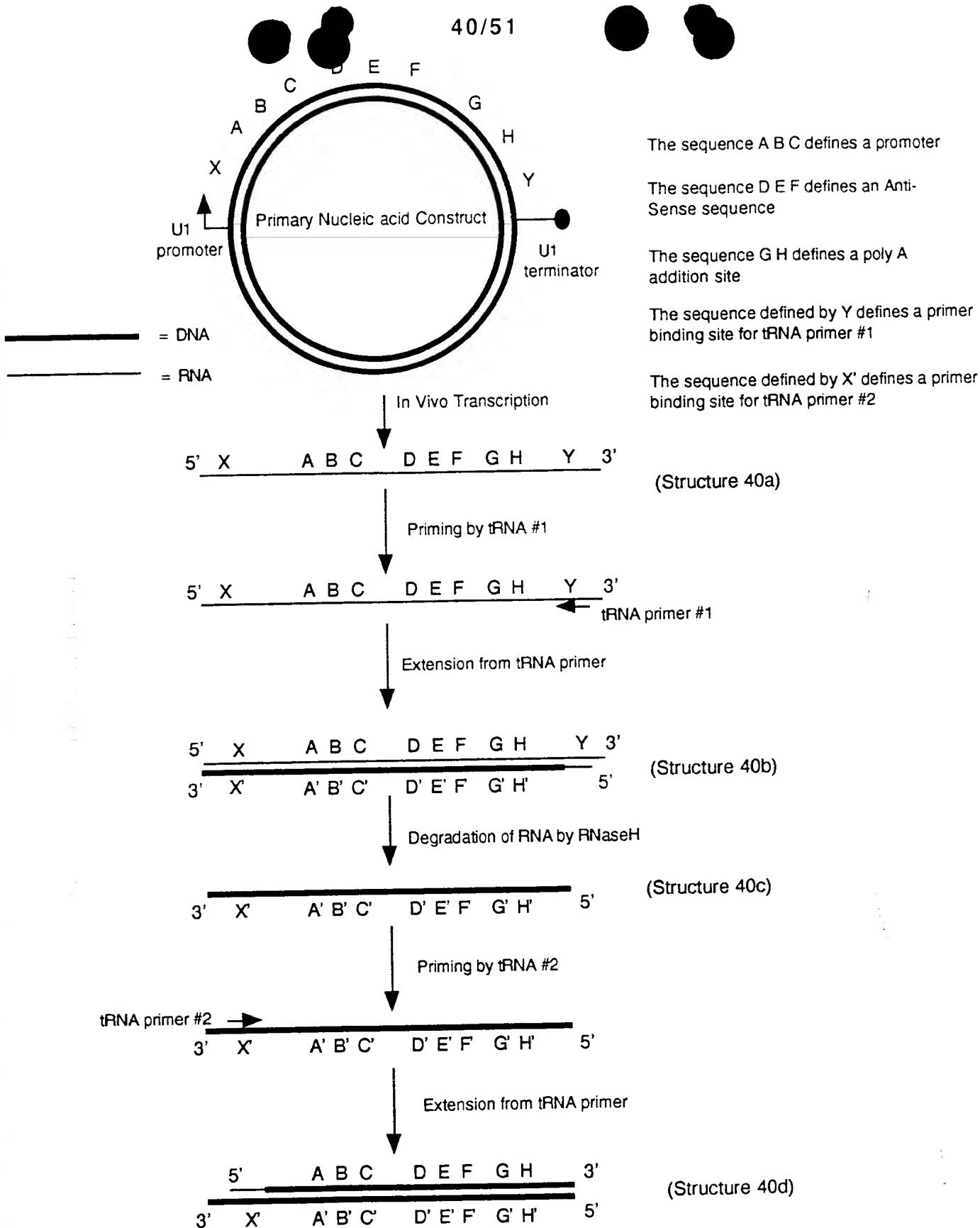
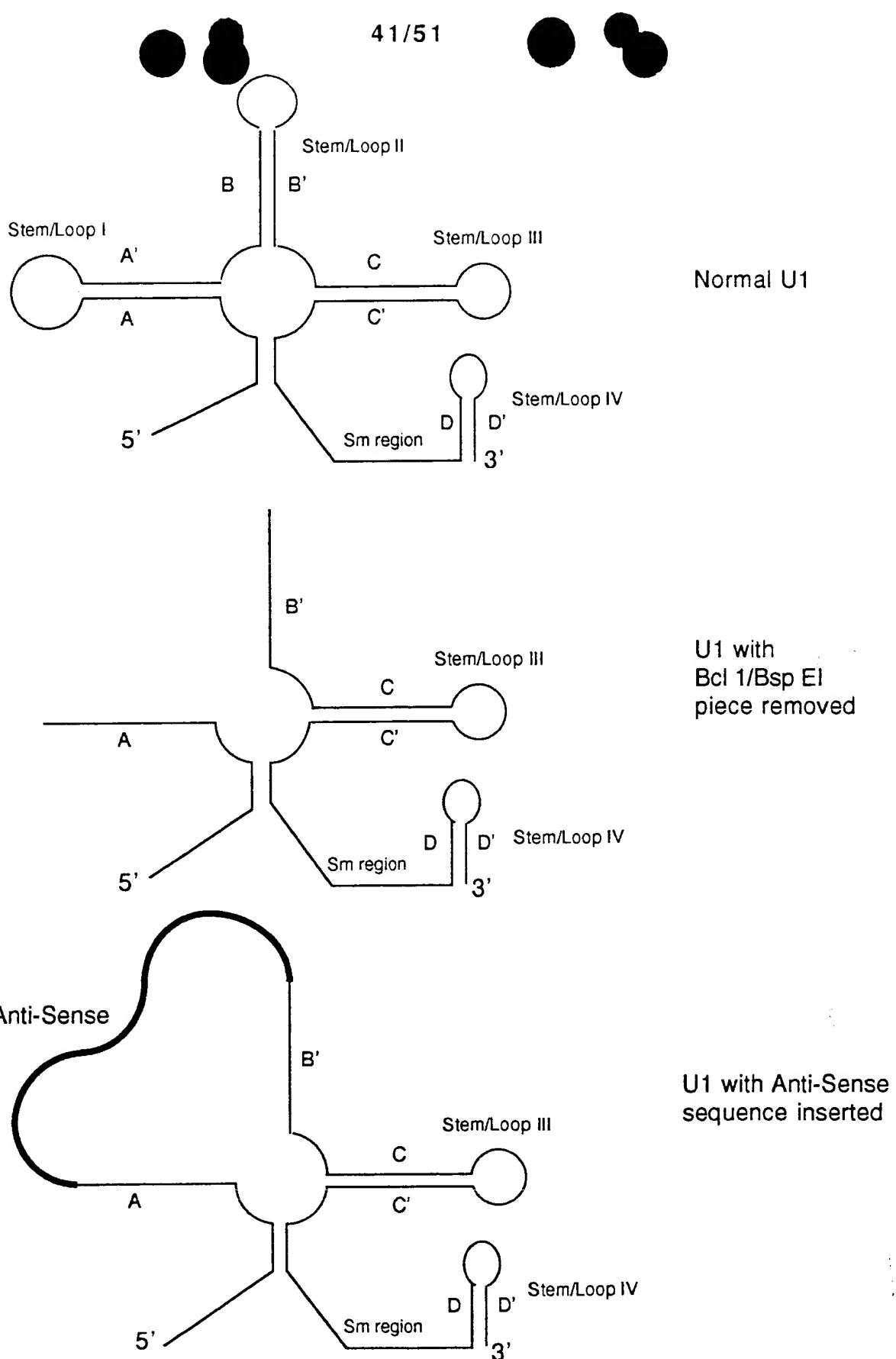


Figure 40

Use of tRNA primers to create a DNA construct for secondary production of transcripts

**Figure 41**

Excision of Sequences from U1 Transcript Region
and Replacement with Novel Sequences

(A) Anti-sense oligomers

HVA-1 GAT CCG GAT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT
 HVA-2 CCG GAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGC TTA AGC CTC AAT CCG
 HVB-1 GAT CCG GAC CTT GAG GAG GTC TTC GTC GCT GTC TCC GCT TCT TCC TGC CAT AGG AGA GCC TAA GGT
 HVB-2 CCG GAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA GGT CCG
 HVC-1 GAT CCG GAT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT
 HVC-2 CCG GAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC ATC CG
 HVD-1 GAT CAG CAT GCC TGC AGG TCG ACT CTA GAC CCG GGT ACC GAG CTC GCC CTA TAG TGA GT C GT A TTA T
 HVD-2 CCG GAT AAT ACG ACT CAC TAT AGG GCG AGC TCG GTA CCC GGG TCT AGA GTC GAC CTG CAG GCA TGC T

(B) Replacement of U1 sequences with HIV Anti-sense sequences

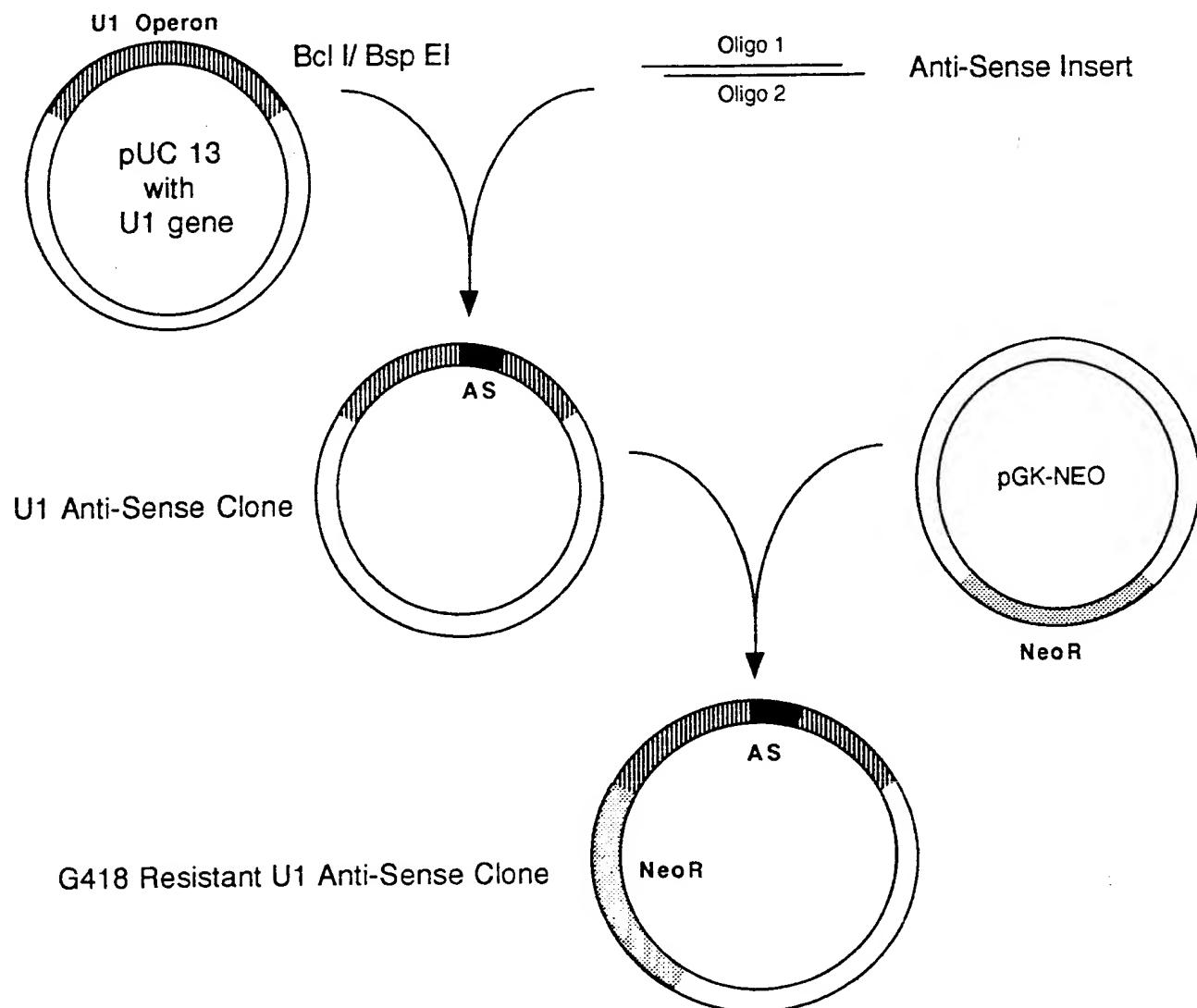


Figure 42
 Insertion of Anti-Sense Sequences into U1 Operons

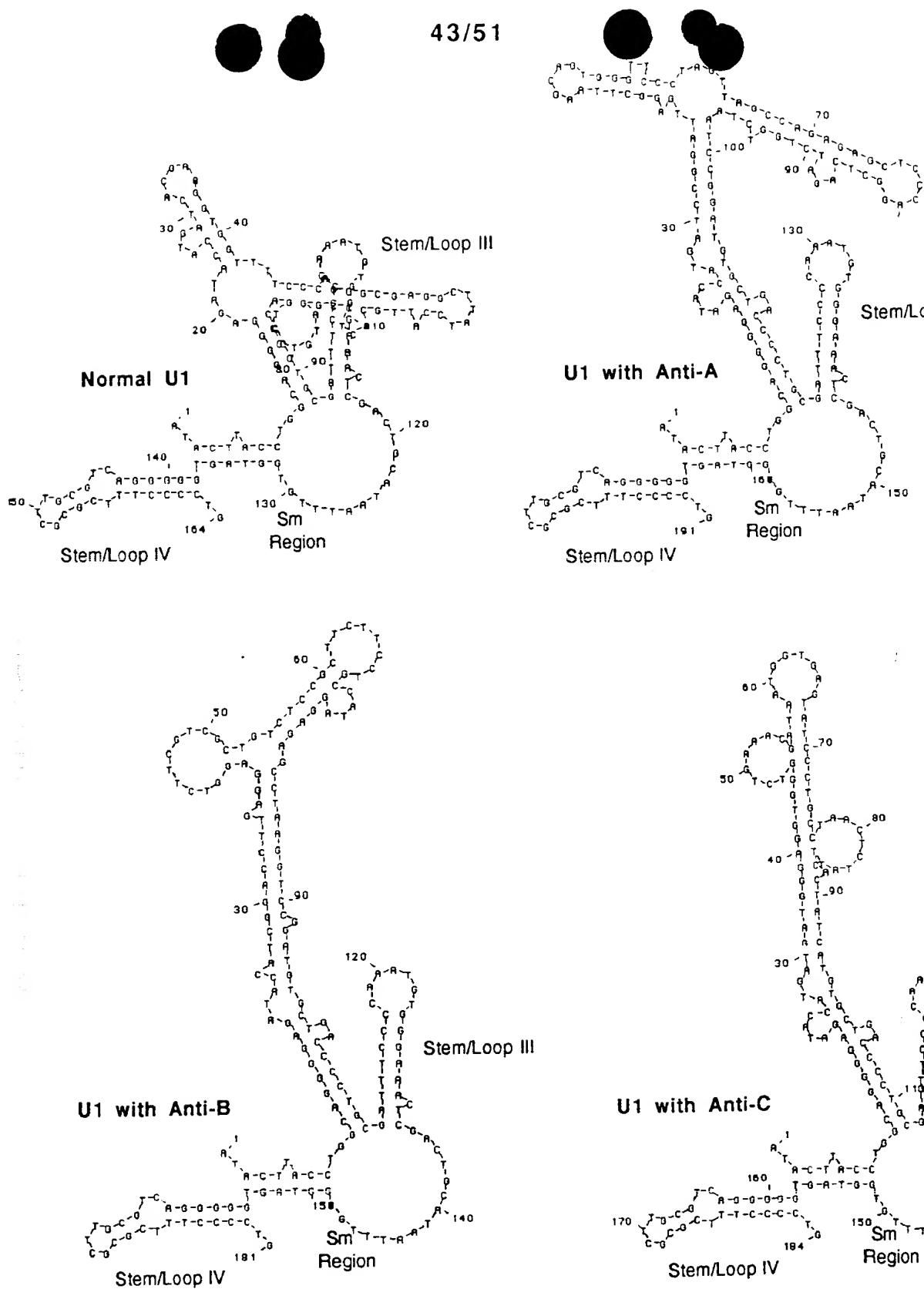


Figure 43

Predicted Secondary structures for U1
Transcripts with Anti-sense Substitutions

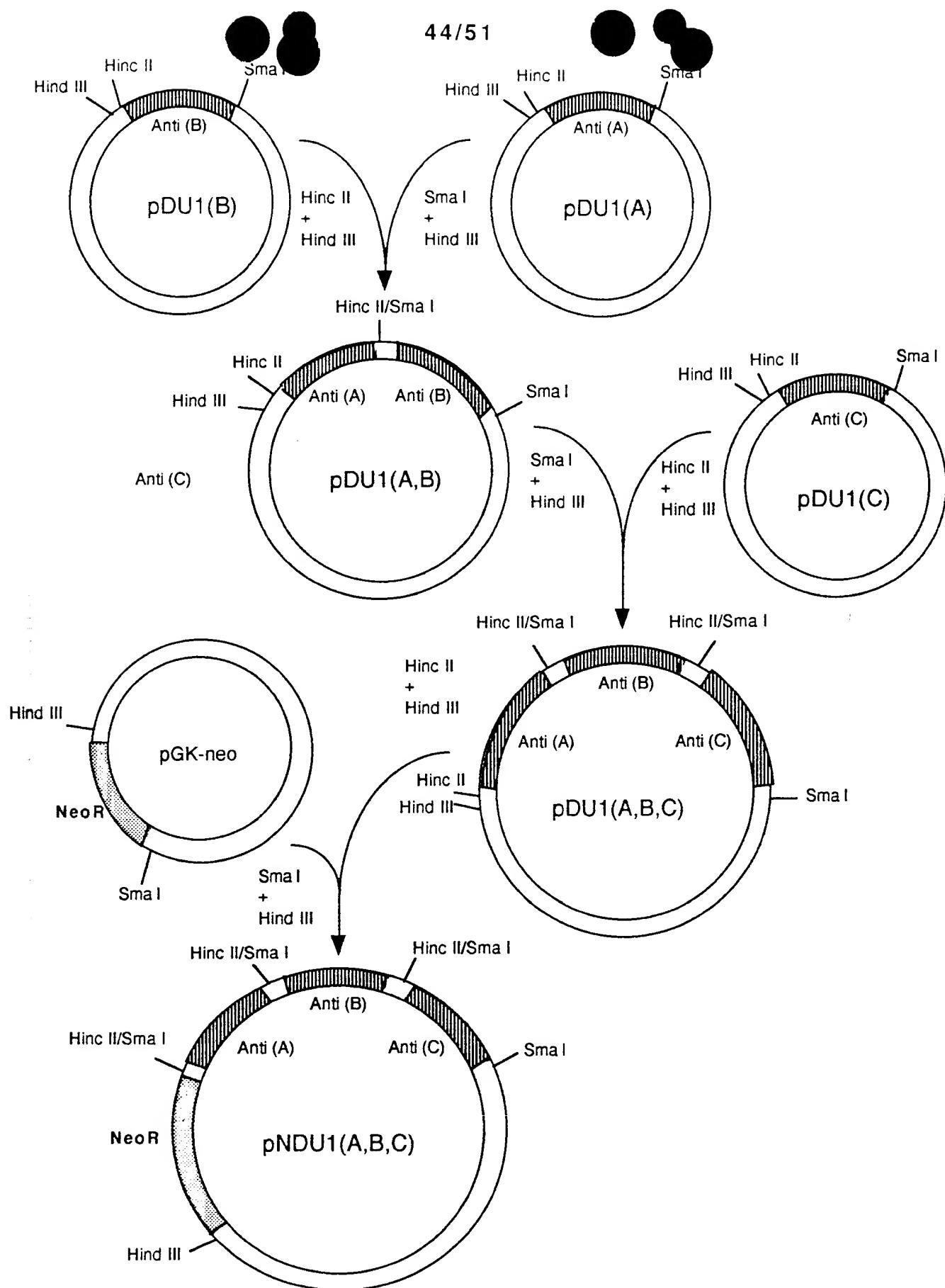


Figure 44
Construction of U1 Multiple Operon Clone

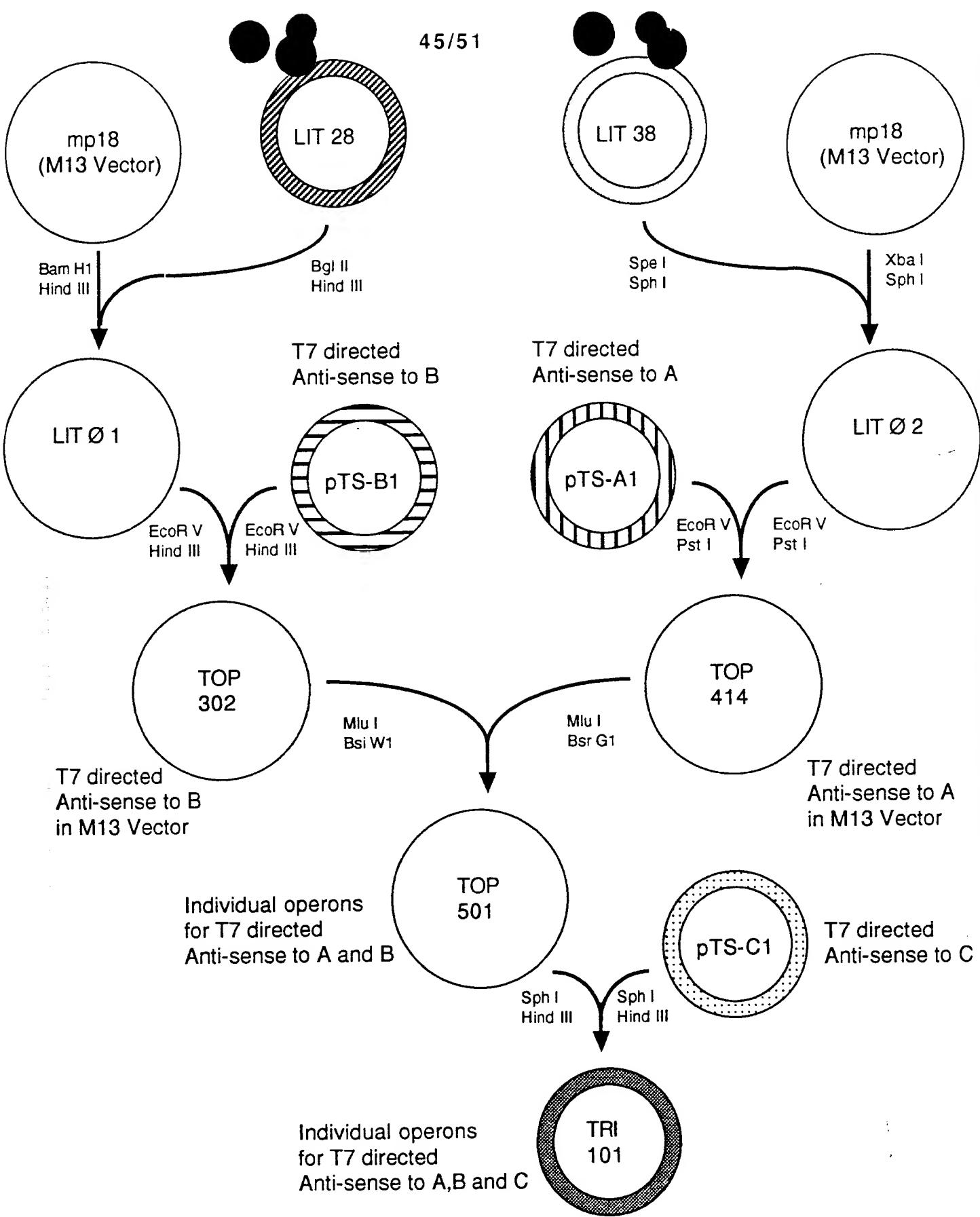
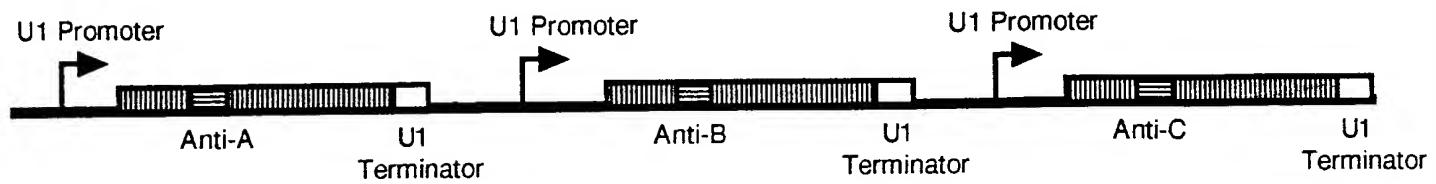


Figure 45
Construction of T7 Triple Operon

pNDU1(A,B,C)

Triple U1 Operon Construct with HIV Anti-Sense



TRI 101

Triple T7 Operon Construct with HIV Anti-Sense

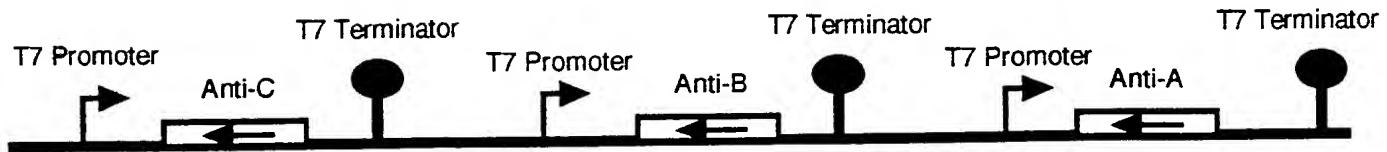


Figure 46

Structures of Triple Operon Constructs
from Figures 44 and 45

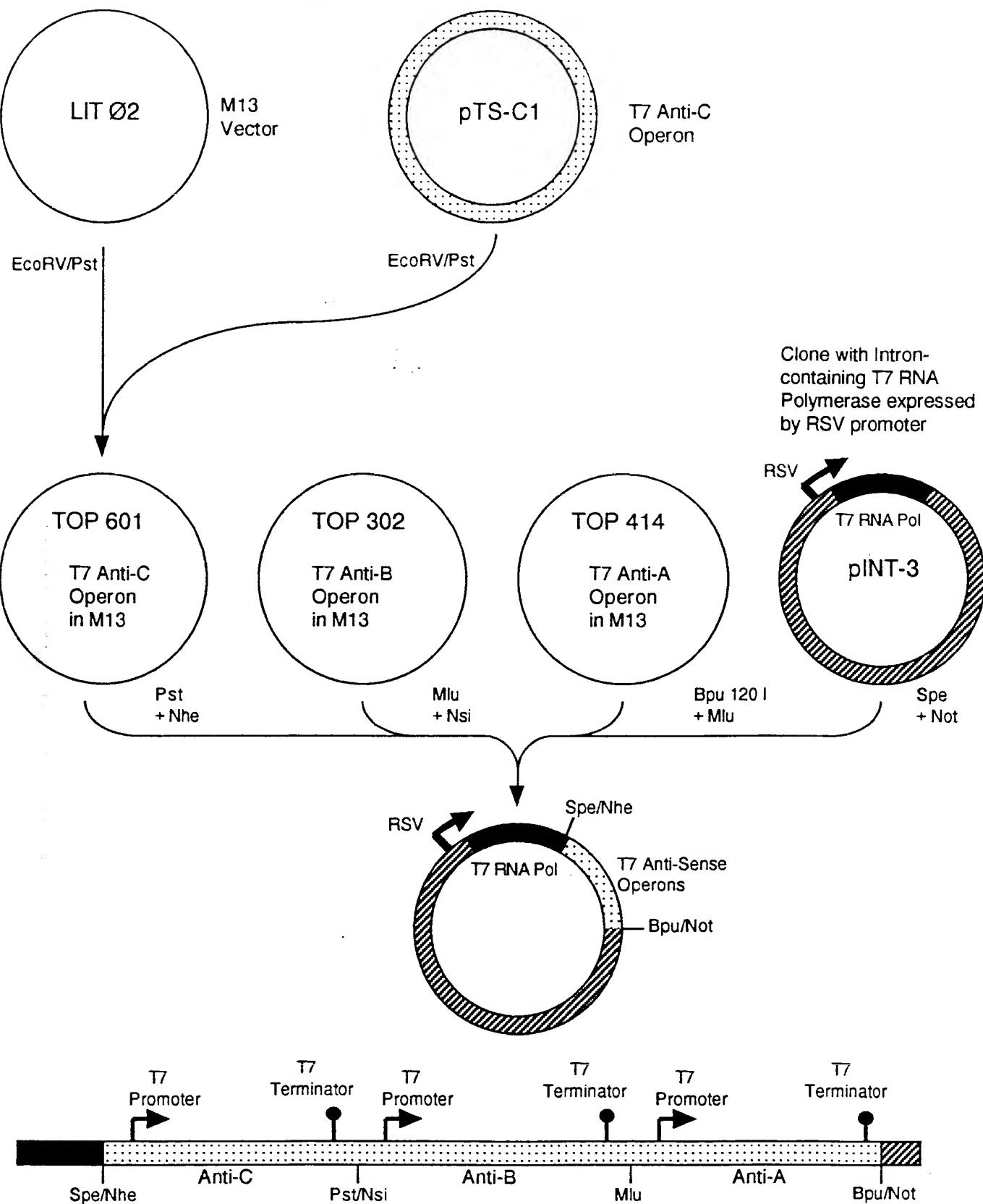


Figure 47
Construction of Multiple T7 Operons in Vector coding for T7 RNA Polymerase

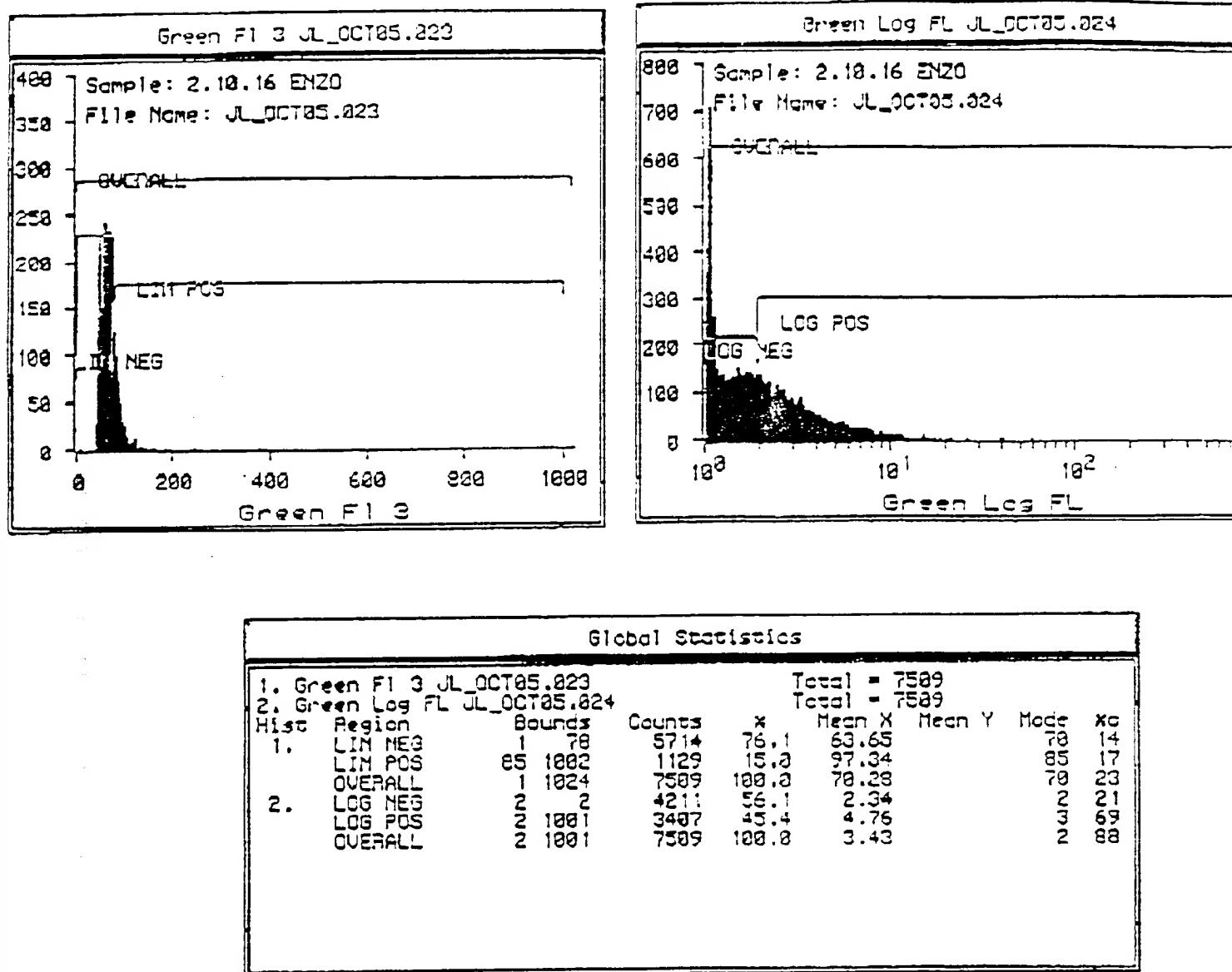


Figure 48

Flow cytometry data measuring binding of anti-CD4+ antibody to HIV resistant U037 cells

49.51



Figure 49

PCR amplification of gag region
indicating absence of HIV in
viral resistant cell line (2.10.16)
after challenge

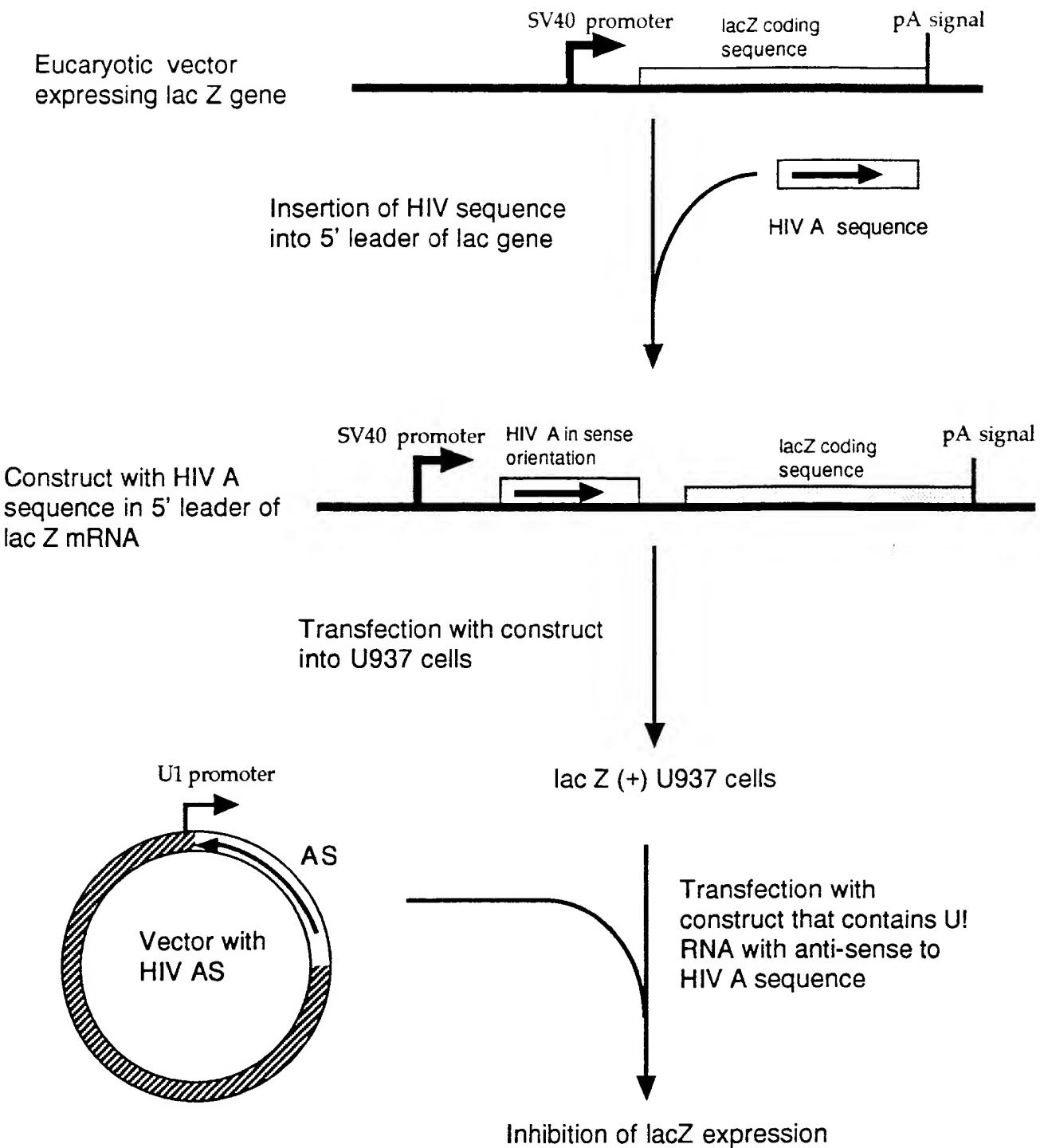


Figure 50

Clone with target-*lacZ* fusion will have reduced expression of *lacZ* after transfection by HIV Anti-sense construct

Enzyme activity as expressed by A_{420} readings
in extracts prepared from

	2.5×10^4 cells	5×10^4 cells	1.0×10^5 cells
U 937 [untransfected]	0.018	0.023	0.034
U 937 [HIV A clone]	0.154	0.277	0.566
U937 [HIV A / Anti-A]	0.010	0.017	0.027
U 937 [HIV A/Anti-ABC]	0.013	0.021	0.035
U 937 [HIV A / Null DNA]	0.120	0.212	0.337

[B] Expression of Beta-galactosidase activity by *In situ* assay :

U 937 [untransfected] no blue spots in cells

U 937 [HIV A clone] blue spots in cells

U 937 [HIV A/Anti A] no blue spots in cells

U 937 [HIV A/Anti ABC] no blue spots in cells

U 937 [HIV A / Null DNA] blue spots in cells

Figure 51

Expression of Beta-galactosidase activity
in extracts